Toxic Effects on Individuals, Populations and Aquatic Ecosystems and Indicators of Exposures to Chemicals*

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1 INTRODUCTION

Biological responses to chemical pollutants that enter the biogeochemical cycles of the biosphere can be sensitive indicators of the presence and impact of those chemicals. For decades, biologists have measured characteristics of aquatic and other communities to assess their well-being. Much of the effort has been an attempt to find "the all-encompassing measure" that will define the damage and point out the cause. With today's emphasis on chemical contaminants and knowledge of the large number of them to be found in surface water, it is easy to blame "toxics" for all undesirable impacts found. However, the presence of chemicals in a body of water does not signify they are having adverse impact, because impact of toxic chemicals on biological systems is a function of time and concentration. Impacts may be reflected as death or physiological dysfunctions in individual organisms, reduction in growth, reproduction in populations, and alterations in structure and functions of ecosystems. In many situations, it is more important to determine the impact than to characterise the chemical contaminant.

This paper presents two research approaches that address problems encountered in evaluating the effects of complex mixtures of chemicals on aquatic systems. The concept of ambient toxicity testing is applied to the impact of effluents in freshwater receiving waters (the concept also applies to saltwater systems), where measurement of toxicity is made without attempting to identify the toxics. Another approach develops structural and functional indices that can be used to evaluate impacts of chemicals on

* The information in this document has been funded wholly or in part by the U.S. Environmental Protection Agency. It has been subject to the Agency's peer and administrative review and approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Methods for Assessing Exposure of Human and Non-Human Biota. Edited by R.G. Tardiff and B. Goldstein

communities maintained under controlled conditions in the laboratory. One approach is concerned with chemicals already in the environment; the other, with developing ecosystem-level indices used to evaluate chemicals before they reach the environment. Also, applicability of laboratory-derived data to field situations is discussed.

Probably more toxicity and exposure data have been developed on individual organisms maintained in the laboratory than on populations and ecosystems combined. For example, a large data base exists on the concentrations of various chemicals lethal to fifty percent of the test organisms within 96 hours (96-hr LC_{50}). Emphasis was placed on these short-term tests, because of the need for data on an expedited basis and because these data are useful in predicting the effect of a chemical on the same or related species when exposed in the field in the same manner as in the laboratory. Longer duration chronic tests that yield information on sublethal effects also are used in this manner. Unfortunately, most laboratory tests were conducted with single chemicals, and do not yield information on how death or sublethal effects in individuals affect an entire population or other parts of the ecosystem or how mixtures of chemicals affect the organisms.

Chemicals may have multiple effects on populations of organisms including mortality, reproductive failure, and productivity. Sensitivity of populations depends upon such factors as age groups and temporal patterns of exposure. Slobodkin (1967) points out the potential relationship between effects on individuals that can lead to genetic changes in populations. One example of the relationship may be found in acquired resistance to pesticides by the mosquito fish, *Gambusia affinis* (Ferguson, 1963).

Evaluations of effects of chemicals on ecosystems are complicated by the multitude of organisms within a given system, their interaction with the physical/chemical aspects of the system, and the many and varied types of ecosystems. These complexities and the role of biota in the function of a healthy ecosystem are expressed by Levin and Kimball (1984): "Natural ecosystems are dynamic assemblages of thousands of species set in a complex physical and chemical environment with which they interact. The biota regulate ecosystem processes and modify the physical and chemical environment. In turn, the environment influences the diversity and nature of the biota. Therefore, even when the focus of interest is at the ecosystem level, it is important that we assess effects of stresses upon the biota and the role of the biota in modifying the effects of stress."

Certain structural and functional indices can be used to evaluate the health (thus the exposure) of an ecosystem. Structural indices in ecosystems include numbers and kinds of individuals, functional roles of individuals, interactions and interrelationships between and among species, and the general trophic composition of a system (Levin and Kimball, 1984). It is through such indices as trophic composition that effects of a chemical on

one portion of a system may be transmitted to and adversely affect another, far removed site. For example, a chemical could selectively affect the food source of a predator fish, and the effect not be evident until the fish disappeared. Functional aspects include such integrative processes as cycling of nutrients, productivity, and decomposition. The significance of these functions is illustrated by the fact that some vital materials are not available to consumer organisms until processed by microorganisms or other decomposers.

In the aquatic environment, classical measurements of toxicity and the concentration of a chemical in the water, sediment, and biota of a body of water do not necessarily yield information required to relate environmental concentration of a chemical to exposure, and exposure to effects. Indeed, when exposure is due to a complex mixture of chemicals, or a mixture of chemicals sorbed to a substrate such as sediment, and concentration and composition of the mixture vary in time and space, the determinations of exposure and relationship of exposure to effects are more complicated. Sometimes, in a practical sense, it is impossible to associate effects with causes. Often, aquatic communities are too poorly understood to study directly and to discern if toxicity causes an impairment. At the ecosystem level of organisation, we simply have not yet determined in every instance what criteria to use to evaluate change, nor have we quantified the types and degrees of change that may alter ecosystems beyond their ability to recover.

2 AMBIENT TOXICITY TESTING

One of the difficult issues in aquatic toxicological concern is the delineation of exposure that occurs in receiving-water situations. There are several reasons for this difficulty beyond the obvious one: insufficient monitoring. In addition, there are extreme variations in dilution as a result of precipitation either locally or far afield in upstream reaches. More recently, the U.S. Environmental Protection Agency (EPA) began moving towards control of toxicity of mixed effluents, rather than attempting to control effluents only on a chemical-by-chemical basis, because most effluents have hundreds to thousands of components and because chemical-by-chemical examination is simply not feasible. A single chemical analysis of a complex effluent can cost tens of thousands of dollars; since no toxicity data exist for most of the materials identified, there is no basis to set needed detection limits to interpret the analytical findings.

Even more exacerbating is that after discharge, the complex mixture is ever changing in regard to the proportion of each chemical in the mixture and, as time passes, components disappear and other substances not originally present appear as a result of degradation. In reality, there is no

feasible way to establish the exposure that occurs even ignoring the varying biological availability of constituents.

Those accustomed to more classical toxicology of single chemicals or simple mixtures and classical exposure assessment methods will label the complex mixture problem hopeless. However, the problem is there to be solved, and so the challenge is to find the best way to deal with it, realizing that no approach will be totally satisfactory. Unlike situations where human life is directly involved, the acceptable risk is probably larger.

Our approach has been based on the fact that measuring the exposure to relate it to toxicity test conditions is not possible; and, therefore, an alternative is to measure what we might call *in situ* toxicity, wherein we allow natural processes to take place—altering the effluent in whatever way they will—and to measure the result. We have called this approach "ambient toxicity testing."

The reader may think of the use of caged test organisms, as we did. But experience soon revealed that this approach had very limited usefulness. Some places of concern were too difficult to reach, thereby too severely limiting observation and maintenance, such as feeding. If access were easy, then vandalism was severe. Still other places were subject to ships, drifting debris, or other hazards.

Our alternative was to sample the water column at points of concern, and to measure the toxicity of the samples collected. The availability of a recently developed 7-day full chronic test made this approach practical because of both time (and, therefore, cost), and the greater sensitivity since it is a chronic test. Heretofore, only acute tests were practical and, while they were short enough, lacked sensitivity.

Fluctuations in contaminant composition are "smoothed out" in receiving water, as compared to effluents, as a result of dilution, flow-time, and mixing. Since we are estimating chronic toxicity, we prefer to use composite samples, where possible, to better simulate exposure. Or in an additional step to improve our exposure simulation, we use a 24-hour composite sample period and seven such samples for the entire test period of seven days, so that the test organisms are exposed to seven different composite samples, each for a 24-hour exposure.

To be sure, we do not define what the exposure was, but we do know it was a reasonable approximation of what actually occurred at the sampling point. Furthermore, because we can control and measure other conditions more easily and accurately in the test conditions, we know the result obtained is due to toxicity and not to predatation or some environmental variable, such as temperature, light, or being hit over the head by a thrown stone, as can be the problem with caged animals.

Convincing evidence of this as a sound approach is the excellent correlation obtained with the measured health of the aquatic community at the location where the samples were collected. We have made this comparison for over

90 stations on 10 rivers or estuaries, and we obtained a correlation significant at the 0.001 level of probability. These sites included large and small streams and one estuary.

If one can take frequent grab samples, or better still, a continuous composite by use of an automatic sampler, the situations difficult to handle (such as estuaries having tidal movements) can be tested quite well, because the sampling procedure integrates the exposure received to a degree not otherwise possible to achieve.

Ambient toxicity testing, using cladocerans (*Ceriodaphnia reticulata*) and fathead minnow (*Pimephales promelas*), has been employed to evaluate the impact of chemical effluents on several streams and rivers. Seven-day chronic tests were developed with these organisms. A three-brood life cycle test for *C. reticulata*, using several techniques, can be completed in seven days, and is applicable to on-site measurements (Mount and Norberg, 1984).

Briefly, the methodology involves culturing *C. reticulata* in the laboratory and exposing them to specific effluents. Tests have shown that 150 adults, fed daily, are enough to produce 60 young every 2 hours. Tests are started with 0 to 2-hour old young, and the test solution can be renewed daily, if desired. After seven days, the number of young produced in test and control solutions are compared statistically. Likewise, in the fathead minnow test, the growth of larvae (as indicated by their weight) in test solution during a seven-day test period is compared with growth of fish in control solutions. On-site ambient toxicity tests with *C. reticulata* were conducted in mobile laboratories to determine the toxicity of receiving waters in the Ottawa River, Ohio (Mount and Norberg, 1984). Seven stations were evaluated with a work load equivalent to one standard test with five concentrations

Table 1. Ambient toxicity in the Ottawa River, Ohio[a]

Stream station	River mile	Mean no. (SD) of young per female[b]
2 (Discharge No. 1)	37.7	29.2 (7.1)
3 (Discharge No. 2)	37.4	2.7 (2.5)
4 (Discharge No. 3)	36.9	11.9 (4.6)
5	35.4	8.4 (5.3)
6	32.6	4.2 (2.6)
7	28.8	6.8 (5.6)
8	16.0	27.9 (3.3)

[a] Mount and Norberg 1984
[b] 10 animals/treatment

and controls. The results of an ambient toxicity test over 22 miles of a stream with three major effluent discharges are shown in Table 1. Thus, by measuring chronic toxicity on-site, in 7 days for seven stations in an 8-hour working day, receiving water toxicity can be assessed without using an application factor or other means of extrapolation.

In another study, ambient toxicity testing utilizing both the cladoceran and fathead minnow evaluated the toxicity of receiving water in Five Mile Creek, Birmingham, Alabama (Mount, Steen and Norberg-King, 1985). The sites were visited in October 1983 when stream flow had been low and stable for several weeks, and nine ambient stations had been established. Stations 3 and 4 were located downstream of Coke plants and station 7 downstream of a publicly owned sewage treatment works (POTW). The following data from the study are presented to show the kind of information that can be derived from ambient toxicity studies.

The ambient stations with the highest production of *C. reticulata* young or best growth of fathead minnows are used as the basis for statistical analysis (Table 2). When comparing all ambient stations with Station 6, only Station 7 was not significantly different based on young production; but Station 7 did have significantly lower survival of *Ceriodaphnia*. Survival was 80 percent or higher at all stations, except at Station 7, where only 20 percent survived. Most of this mortality occurred on Day 5, with some on Day 6. This pattern suggests that a discharge of high toxicity of short duration may have occurred, causing the effect at Station 7.

In ambient tests with fathead minnows, weights of fish at Stations 5 and 6 were significantly lower ($P < 0.05$) than at Station 2. Survival was significantly lower only at Station 5 ($P < 0.05$).

Table 2. Survival and mean young per female *Ceriodaphnia* after seven days of exposure to water from various ambient stations, Birmingham, Alabama, October 1983[a]

95% Sampling station	Percent survival	Mean Number of young per female	Confidence intervals
1	80	14.4[b]	9.8–19.1
2	100	18.1[b]	16.5–19.7
2A	100	21.3[b]	19.6–23.1
3	100	20.1[b]	17.5–22.7
5	100	22.4[b]	18.7–26.1
6	80	35.6	30.4–41.0
7	20	29.3	24.6–33.1
8	90	22.1[b]	18.8–25.7
9	100	22.5[b]	18.9–26.1

[a] From Mount *et al.* 1985

[b] Significantly lower than the reference Station 6 ($P < 0.05$)

The fathead minnow ambient toxicity data differ from the *Ceriodaphnia* data. There was no evidence of toxicity to the fathead minnows at Stations 1, 2, or 2A; but *Ceriodaphnia* young production was reduced. However, Stations 5 and 6 were the only ambient stations at which significant toxicity to fatheads was measured, but daphnids showed no toxic effect at Station 6 and very little at 5.

In ambient toxicity tests, the conventional view of a control must be changed. One never knows if any chosen station is free of toxicity. Therefore, no station can be considered a control. A pseudo-control is run using some other water in which the performance of the animal is known. If the performance obtained in this water is within expected ranges, the animals are judged to be in acceptable condition. However, we do not expect the absolute performance to be the same in the "control water" as it would be in the test water, so statistical differences are not based on the "control" but rather on the sample having the best performance. Since our interest here is relative toxicity rather than absolute toxicity, this approach is acceptable.

These two studies indicated the applicability of ambient toxicity tests to evaluate the toxicity of effluents in river systems, and illustrated how different species have different sensitivities. Results of these tests give a comparative toxicity of various parts of the river, and point to areas that may require additional testing. The results also were similar to the biological impact found in the stream.

3 SYSTEM LEVEL EFFECTS

Often, it is necessary to predict the impact of chemicals and other materials on the aquatic environment before measurements of fate and effects can be made in the field. In these instances, it is important to have laboratory test systems that represent the potential sites of impact in the field and to have some quantitative information on that representation. The following study indicates that predictive studies with complex mixtures of chemicals (a drilling fluid) and communities of organisms can yield useful exposure and effects data.

Effects of a used drilling fluid on an experimental seagrass community (*Thalassia testudinum*) were measured by exposing the community to the suspended particulate phase (SPP) in laboratory microcosms (Morton *et al.*, 1985). Structure of the macroinvertebrate assemblage, growth, chlorophyll content of grass and associated epiphytes, and rates of decomposition (as indicated by weight loss of grass leaves) in treated an untreated microcosms were compared. Health of plants and structure of the macroinvertebrate assemblage maintained in the laboratory were compared with the seagrass community from which the plants and attendant communities were taken.

Seagrass communities are excellent assemblages of plants and animals to work with as experimental communities. These productive communities are potentially subject to exposure of chemicals contained in dredged material, oil spills, and from other point and non-point sources. Seagrasses can be maintained in the laboratory and are amenable to ecological measurements, such as those mentioned above.

Drilling fluids are a complex mixture of chemicals, clay and water. The fluids usually consist of bentonite and other clays, barium sulfate as a weighting agent, lignosulfonates as thinners, and other chemicals added for special purposes. For example, diesel oil has been added to some fluids to lubricate the bit. Drilling fluids are injected into the bore-hole of a well to transport cuttings produced as the bit drills through various substrates. The fluid also lubricates the bit, forms an impermeable layer on the inside of the bore-hole to maintain formation pressure, and performs other functions. Drilling fluids are recirculated, when possible, but discharged into surrounding waters when they no longer perform the intended functions.

The experimental design and methods used in this experiment are described in detail by Morton *et al.* (1985) and Price *et al.* (1985). In general, portions of a seagrass community were brought into the laboratory, exposed to drilling fluid or montmorillonite clay, harvested and the data subjected to various analyses. Samples of *Thalassia* were collected by divers with a coring device that penetrated to a depth of about 10 cm. The core was placed intact inside a clear cylinder (16 cm diameter and 50 cm in height). Thus, each microcosm contained *Thalassia* plants, epiphytes, and natural sediment substrate with associated organisms. Microcosms were maintained in flowing seawater and exposed to either drilling fluid or montmorillonite clay; control microcosms were exposed to flowing seawater only. The clay exposure served as a sediment "control" so that any effects of the drilling fluid, a mixture of sediment and solids, could be identified as chemical or physical effects. There were 16 replicate microcosms for each treatment and control.

The drilling fluid used in this study was collected from an operating oil rig in the Gulf of Mexico and contained approximately the same concentration of metals as did other fluids collected at about the same time. However, the fluid used in this experiment contained more diesel oil than did most others. Chemical composition of the fluid is described in detail by Duke and Parrish (1984). The fluid contained relatively large amounts of nine trace metals. The SPP fraction of the fluid and clay mixture was used and an average of 190 microlitres per litre was delivered to the microcosms. This resulted in 15 milligrammes of solids per litre of water. The concentration of drilling fluid simulated the amount that a seagrass bed located near the discharge point might receive.

A leaf litter or detritus bag was added to each of the 48 microcosms. The weight of the dried *Thalassia* leaves in each bag was determined just before

the bags were placed in the microcosm, and at the termination of the experiment to determine weight gained or lost during the experiment.

Each microcosm was harvested at the end of the 42-day test. *Thalassia* leaves and associated epiphytes were taken from each treatment for analysis of biomass and chlorophyll content. Sediments were seived, macroinvertebrates sorted and identified, and the ten species with the greatest number of individuals were designated "dominant" species.

4 EFFECTS ON COMMUNITY STRUCTURE

Exposure to drilling fluid or clay for 42 days significantly affected the structure of the invertebrate community in microcosms. Indices for structural effects included significant differences (at the $P = 0.05$ level) in numbers of the ten numerically most dominant individuals in control and treatment microcosms. There were significantly fewer individuals of four dominant species in microcosms exposed to drilling fluid or to clay than in the control. Three species were significantly affected by both drilling fluid and clay. Perhaps the most striking effect observed was the overall reduction of the number of dominant individuals per microcosm in drilling fluid and clay treatments. Treated microcosms contained approximately one-half the number of individuals as control microcosms. Since the structure of the community was adversely affected by both drilling fluid and clay treatments, the effect was probably the result of alteration of the particle size of the sediment substrate or a physical "layering" effect (the drilling fluid and clay formed a 1 to 2 cm layer over the sediment in the microcosm after the 42-day exposure).

5 EFFECTS ON COMMUNITY FUNCTIONS

The drilling fluid and clay adversely altered some aspects of production and health in *Thalassia* and epiphytes. Plants treated with drilling fluid and clay showed significant reduction in chlorophyll *a* per dry weight of leaf tissue. Also, significant reductions occurred in epiphyte biomass and an epiphytic photosynthetic potential (chlorophyll *a* per cm^2) in drilling fluid treatments. Epiphytes exposed to drilling fluid and clay contained similar amounts of chlorophyll *a* as epiphytes in the controls at the end of the experiment.

The drilling fluid, but not the clay, caused a significant decrease in the rate of decomposition of seagrass, as estimated by weight-loss of the litter bags. The authors interpreted the significant difference between weight loss of leaves in litter bags exposed to drilling fluid and weight-loss of those exposed to clay and seawater (the drilling fluid-exposed bags lost less weight) as an indicator of the toxic effect of drilling fluid on rate of decomposition.

Since the clay did not affect decomposition of *Thalassia* leaves, the effect was considered toxic rather than simply physical. The adverse impact on the decomposers in this system is important, because they perform a vital, yet vulnerable, function for the seagrass community. The seagrass beds require the decomposition of leaves that have broken off and fallen to the bottom or have been clipped off by grazers to form the proper anerobic substrate for growth and propagation. The decomposed organic material also is consumed, or at least the bacteria and the detritus are consumed by seagrass inhabitants.

These type experiments are rather labor- and time-intensive. The microcosms were exposed for six weeks, and another three weeks were required for harvesting the communities, preparing the microcosms and other apparatus, and measuring the various indices. The microcosms did, however, provide a vehicle for measuring ecosystem level effects in an experimental environment.

6 FIELD VALIDATION

It is important to establish the uncertainty of data collected in the laboratory in relation to the applicability of the data to field situations. Rather than "validation," this process often determines the limits of applicability of laboratory data used to extrapolate results to the field. In the case of ambient toxicity testing, validation can mean comparison of ambient toxicity data to instream community effects. Knowledge of the uncertainty (thus the certainty) of laboratory data is especially important when the data are used to predict environmental concentrations (exposure) and effects in risk assessment processes.

Data obtained through ambient toxicity testing in the Five Mile Creek study compared well with those obtained through instream biological measurements such as community loss index (number of zooplankton, benthic macroinvertebrates and fish taxa present). Although use of numbers of taxa present as indicators of the health of a community presents some difficulties in interpretation, it does serve for comparative purposes in this instance.

The trends of percentage increase in toxicity and percentage reduction in taxa were compared. An initial level of 20 percent toxicity was selected, because it represents the maximum control mortality usually accepted for the laboratory tests longer than 96 hours in duration. A range of percentage reductions from 20 to 80 percent was examined for trends between laboratory and field data. An attempt to attribute impact to any or all of the three discharges was not made. Using 20 percent increase in toxicity, 87.5 percent of all stations that used either 20 or 40 percentage reduction levels for the field data were correctly predicted. However, using 40 percent increase in

toxicity for the laboratory data, a poorer prediction for any level of field impact was obtained. Both 60 and 80 percent levels for toxicity data gave 87.5 percent of the stations correctly predicted at the 80 percent level for field data. However, this is all correct prediction of no impact, since neither the toxicity nor the field impact was that severe.

In the seagrass experiment, it was not possible to measure the impact of the drilling fluids *in situ*; however, the health of the seagrass community maintained in the laboratory was compared at the termination of the experiment (42 days exposure) with the field community from which it was collected. Density of the ten numerically dominant species, concentrations of chlorophyll *a* of *Thalassia* leaves and epiphytes and ash free dry weight of the epiphytes were compared. Among the ten most abundant genera in laboratory control microcosms and field cores, six were common—four annelids, one arthropod, and one mollusc. Seven of the ten laboratory control genera were found among the total genera identified from the field cores, and all top ten dominants in the field were present in all the genera identified in laboratory control microcosms. The only significant difference between laboratory and field in other measured characteristics was chlorophyll *a*, which was higher in laboratory-maintained epiphytes and in *Thalassia* leaves in laboratory microcosms. The elevation of chlorophyll *a* could be a natural reaction to less light since laboratory microcosms received from 50 to 80 percent less light than did the field communities on a clear day. It is reasonable to assume that seagrass communities removed from the field and maintained in the laboratory for six weeks will vary in structure and functions from undisturbed systems. These data helped to quantify that difference.

These results indicated that the microcosms were reasonably similar structurally and in measured functions. It appears valid to compare the more general aspects of structure and function when extrapolating data obtained in these benthic microcosms. For example, the effect of the drilling fluid on total numbers of organisms should be considered, rather than the effect on one particular genus or species.

7 SUMMARY

Ambient toxicity testing is a viable means for establishing the toxicity of mixtures of chemicals to aquatic organisms, and results to date compare favorably with instream measurements of community health. Naturally, one or two organisms will not be sensitive enough to detect all contaminants at low concentrations. No measurement is able to find every problem every time. In tests conducted thus far, ambient testing has revealed more about chemical contamination than any other single measurement and at less cost.

Indices of ecosystem-level effects were developed that reflect structural and functional impairment of aquatic communities by complex mixtures of

chemicals, even when the chemicals are adsorbed to, or at least associated with, clays (drilling fluids). Indices such as numbers and kinds of organisms, primary productivity, growth, and decomposition proved to be useful in this regard.

Where possible, it is important to compare results obtained in the laboratory with those obtained in field situations. In those instances when predictive laboratory tests with communities are involved, the health of the test systems should be compared with the health of a similar community in the field. Such comparisons made during development of the two research approaches reported in this paper are encouraging.

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