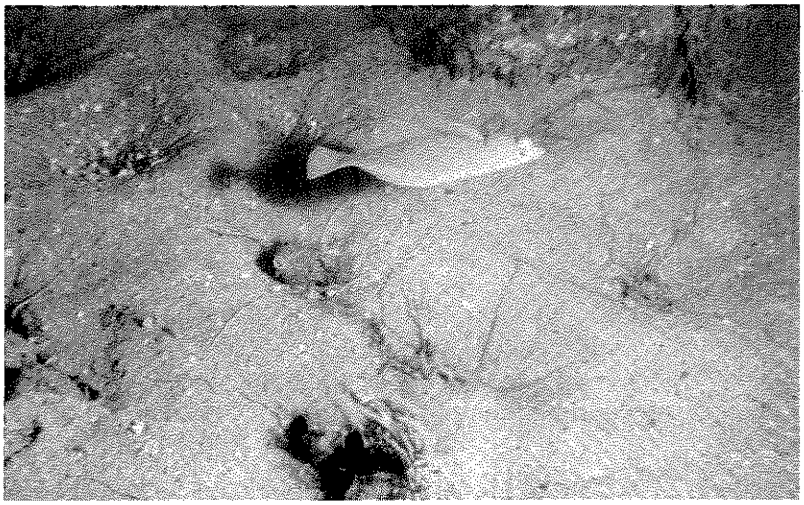

REVIEW OF BIOASSAY TECHNIQUES

In a 130-page report, David A. Brown, Steven M. Bay, and Bruce E. Thompson review a variety of bioassay techniques available for evaluating the effects of contaminants. The researchers assessed bioassays reported in the scientific literature and paid particular attention to standard bioassays of the American Public Health Association, the Environmental Protection Agency, and the American Society for Testing of Materials. Criteria for review also were to include as many sublethal assays as possible and to focus on marine and aquatic bioassays. In all, 190 articles or books are cited in the reference list. The purpose of the review is to provide a comprehensive reference for the technical personnel of sewage and other water quality agencies.

For the purposes of the review, the researchers divided the bioassays into three major categories according to the location of the exposures as follows: laboratory exposures, field exposures, caged-field exposures. The term bioassay was used in its broadest sense; that is, as a measurement of any perturbation of a biological system. Responses reviewed included bioassays of population/ community changes, survival, growth, reproduction, behavior, performance, bioaccumulation, histological changes, and physiological and biochemical responses. Physiological responses included respiration, oxygen:nitrogen ratio, scope for growth, lysosomal latency, and luminescence (Microtox). Microtox is a commercially-produced bacterial luminescence test which has been shown to correlate well with marine larval invertebrate bioassays. Biochemical measurements included the hormonal stress response, the bioenergetic stress response, detoxication/toxication mechanisms, adenylate energy charge, enzymatic effects, genetic effects, immunological responses, blood chemistry responses, and free radical generation. Several biological levels of organization were reviewed in hopes of demonstrating a cause and effect relationship between bioaccumulation and organismal or population/community effects.

The following discussion of Brown, Bay and Thompson's findings is adapted from the "Conclusions and Recommendations" section of the review.

Acute toxicity bioassays on adult organisms provide little information on effects of effluents in receiving waters because acutely toxic effects seldom occur in the natural environment. However, bioassays of fertilization success, hatching success, and early embryonic development may be relevant to effects in the receiving environment since recent studies near both Los Angeles and San Francisco have shown that present environmental concentrations of contaminants are within the range which impair reproductive processes. Thus, it is recommended that development of bioassays for purposes of predicting effects upon organisms in the receiving environment focus on reproduction and early development. These bioassays should be kept as realistic as possible with regards to routes of exposure through particulates, through the sediments, and through the food chain. For instance, flatfish should be exposed using a layer of sediments that contain a food source such as polychaetes.

For purposes of economy, it is recommended that short-term toxicity tests be examined to determine whether these provide results that can be used to predict the results of long-term life cycle tests. This will require that long-term studies of growth and reproduction be calibrated with short-term tests. For example, studies are presently being conducted by the Environmental Protection Agency to compare 7- and 28-day fish survival and growth. Another candidate for such testing is SCCWRP's 48-hour sea urchin fertilization and development test.

Although short-term toxicity tests such as the Microtox bacterial luminescence test have little ecological relevance, they may be useful in some circumstances. For instance, Microtox is useful for determining sources of toxicity in influent streams, monitoring of toxic levels in influent streams so that kills of effluent-treatment organisms can be avoided, and monitoring effluent treatment success. Microtox has the advantage of being the most rapid (30 minutes) toxicity test of those reviewed. If Microtox toxicity values can be clearly related to environmentally relevant parameters, such as reproduction in other organisms, then they might be useful for routine monitoring of effluents. Comparisons between Microtox and marine invertebrate bioassays with the same sediments from Puget Sound have shown a good correlation.

In order to relate organismal effects on survival, growth and reproduction to effects on populations and communities it would be necessary to conduct mesocosm experiments similar to the Controlled Ecosystem Pollution Experiment (CEPEX) and the Marine Ecosystem Research Laboratory (MERL). However, large-scale bioassay systems like CEPEX and MERL are not practical for routine toxicity research programs. If mesocosm experiments are conducted, exposure tanks should be similar to the MERL enclosures since these utilized a sediment phase. The system should be designed to include several replicate tanks per exposure to avoid the problem of pseudo-replication.

One of the most troublesome concerns with regard to bioassays is the interpretive extrapolation from laboratory exposures to anticipated effects in the field. Thus, it is necessary that laboratory results be validated by field observations. The likelihood of field observations validating laboratory results should increase as the similarity between laboratory exposure regimes and the field increases. However, it may not be possible for laboratory assays to account for all influences on physiological parameters which may be present in the field. Therefore, field-oriented toxicity tests need to be developed. Because of the multitude of contaminants present in the field, field-oriented bioassays need to include contaminant-specific assays of toxicity such as detoxication/toxication assays. During laboratory development, these contaminant-specific assays should be calibrated with important physiological parameters such as survival, growth and reproduction so that observed changes of these parameters in field-exposed organisms can be related to the degree of detoxication or toxication of specific contaminants.

Some of the most important monitoring will be that conducted in the receiving environment. The use of community structure and species composition measurements to detect changes in biological conditions due to discharge are generally well accepted and are currently part of most marine environmental monitoring programs. Measurements of population and community parameters may be thought of as *in situ* bioassays since results can be used as a test of environmental quality. However, although such programs may show changes in environmental quality, the causative agent for observed changes is often difficult to determine. This emphasizes the need for development of field-oriented contaminant-specific assays of toxicity.

Because of the large historical discharges of contaminants that have taken place in southern California, it will be difficult to distinguish effects of present day effluents from past discharges in the receiving environment. For this reason, it is recommended that laboratory bioassays be conducted to test the relative contributions of present-day effluents and historical discharges to present-day contamination. The major source of contaminants from historically-released contaminants in southern California is thought to be sediments, based on evidence from measurements of the DDT to PCB ratio. This ratio, which used to be greater than 2:1 in southern California sewage effluent, has over the past 15 years decreased to about 1:7. Recently, researchers found that the closer to sediments that organisms are caged in the water column the higher the bioaccumulation of contaminants and the higher the DDT/PCB ratio. Finding high DDT/PCB ratios in organisms suggests that they are most affected by sediments, which are slow to reflect the changes in input of DDT and PCB in discharges. Thus, it is recommended that bioassays designed to distinguish effects of past versus present discharges contain the following treatments: (i) clean sediments/clean seawater; (ii) clean sediments/seawater-diluted effluent; (iii) contaminated sediments/clean seawater; (iv) contaminated sediments/ seawater-diluted effluent. Measurements in exposed organisms should focus on bioaccumulation, reproductive success, histological changes and physiological and biochemical indices of toxicity.

It is important that both laboratory and field tests carefully examine and calibrate changes against both circadian (daily) and circannual (yearly) biorhythms. A good example of the importance of seasonal reproductive cycles on tissue levels of contaminants can be found in a report by J. N. Cross (this volume, p. 48). These biorhythms will be one of the largest sources of variability with regards to effects at all levels of organization from the molecular to the population/community. No parameter will be without marked circadian and circannual rhythms. However, if these cycles are clearly defined, then results can be calculated back to a standardized baseline.

Variability in field-exposed organisms will also result from the fact that fish and other organisms may move around so that organism exposures will vary. One approach used to provide a controlled exposure in the field is the use of caged organisms. However, most research has indicated that the stress of caging eclipses any effects of effluent. Thus, caging is not recommended for most organisms. Variability is most effectively dealt with by use of proper statistical sampling design.

STATEMENT OF REVENUE
FOR THE PERIOD ENDED DECEMBER 31, 1986

		Amount
Interest		**$6,080.40**
Aid from other Governmental Agencies		
Joint Powers Agreement:		
County Sanitation District No. 2 of Los Angeles County	$264,974.00	
City of Los Angeles	305,106.00	
County Sanitation District No. 1 of Orange County	171,570.00	
City of San Diego	104,116.00	
City of Oxnard	14,234.00	**$860,000.00**
Associate Member:		
Encina W.P.C.F.	11,690.00	
South East R.R.A.	11,690.00	**$23,380.00**
Contract Service:		
State Water Resources Board	$31,680.00	
Occidental College	400.00	
U. S. Dept. of Commerce	6,000.00	**$38,080.00**
TOTAL		**$927,540.40**

BALANCE SHEET
FOR THE PERIOD ENDED DECEMBER 31, 1986

	GENERAL FUND	FIXED ASSET GROUP OF ACCOUNTS
ASSETS		
Cash with Los Angeles County Treasurer	$56,137.57	
Imprest Cash	8,000.00	
Investments	300,000.00	
Equipment at Cost		$396,196.15
TOTAL	**$364,137.57**	**$396,196.15**
LIABILITIES AND FUND BALANCE		
Encumbrances	$82,044.06	
Fund Balance	282,093.51	
Investment in Fixed Assets		$396,196.15
TOTAL	**$364,137.57**	**$396,196.15**