Chesapeake Bay Ambient Toxicity Assessment Workshop Report

Workshop held
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Editor

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Foreword

The 1983 USEPA report, *Chesapeake Bay Program Technical Studies: A Synthesis*, which detailed the findings of the seven year study of Chesapeake Bay, found high concentrations of metals and organic compounds in some portions of the Bay, most notably in highly industrialized areas such as the Patapsco and Elizabeth Rivers. High levels of metal contamination were also discovered in sediments in the upper mid-Bay area, upper Potomac, upper James, and small sections of the Rappanhannock and York Rivers. In light of these findings, the link between these toxic conditions and the impact upon the Bay's living resources became an important priority in the formulation of the Chesapeake Bay Basinwide Toxics Reduction Strategy. The authors of the Strategy recognized that "no critical compendium of scientific information relating to distribution and effects of toxics in the Chesapeake Bay has been formulated. Without such information, developing hypotheses concerning effects of toxic substances on biota in Chesapeake Bay is difficult if not impossible". They also noted that the link between ambient toxicity assessment and its impact on living resources was not clear enough to direct appropriate management actions.

The Ambient Toxicity Assessment Workshop was convened with the goal of describing the state of the art in the use of biological indicators. As with any emerging field, it is clear that dealing with toxics is highly complex and more research and experience must be gathered. Creative and innovative methods need to be tested and existing techniques need further refinement. Although gaps still remain in our understanding of the effects of ambient toxicity on living resources, enough knowledge has been gained to lay out many important principles relating whole organism toxicity testing, sediment toxicity testing, and to lesser degrees suborganism toxicity testing and population risk assessment (based on toxicity testing).

This document is a first approach to develop consensus protocols for the use of biological indicators to monitor the effects of toxic contaminants in Chesapeake Bay habitats important to living resources. As previously stated, the Chesapeake Bay Basinwide Toxics Reduction Strategy, which was approved and adopted by the US Environmental Protection Agency, the Commonwealths of Virginia and Pennsylvania, the State of Maryland, the District of Columbia, and the Chesapeake Bay Commission, contains a number of commitments in the area of research, monitoring, and toxics management that are necessary to achieve a comprehensive approach to reduce toxics input to Chesapeake Bay.

In recognition that research and monitoring programs will provide new information about the toxics problem in the Bay, the developers of the Strategy made the following commitment:

> "By July 1989, the signatories agree to convene a scientific workshop to develop consensus protocols for the use of biological indicators to monitor the effects of toxic contaminants in Chesapeake Bay habitats important to living resources."

This workshop was a fulfillment of this commitment.

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This workshop would not have been possible without the contributions of a great number of resource managers, administrators, scientists, and technicians. Gratitude is also extended to all participants and supporters, including the workshop's sponsors, steering and planning committees, speakers, conveners, workgroup chairs, recorders, attendees, and those providing financial and logistical support. The names of contributing individuals appear either below or in Appendix B.

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Workgroup Participants
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Workshop Staff

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FINAL PLENARY SESSION

EXECUTIVE SUMMARY

The Ambient Toxicity Assessment Workshop provided a forum on how to use biological indicators to monitor the effects of toxic contaminants in Chesapeake Bay habitats important to living resources.

Why conduct an Ambient Toxicity Assessment Workshop? Many resource managers must assess the impacts of toxics on living resources. It is conceptually attractive to use biological indicators of actual toxicity, rather than measured levels of chemical compounds to infer levels of toxicity. Current bioassessment test protocols, however, often result in complex and conflicting test results. This problem prompted the developers of the Chesapeake Bay Basinwide Toxics Reduction Strategy to call for a toxicity assessment workshop. Its purpose was to provide resource managers with the necessary information to better assess and evaluate the significance of toxic contaminants as causes of mortality and impaired growth and reproduction of Bay organisms.

A three-day workshop of scientists, technicians, and resource managers from the Chesapeake Bay area and around the nation met to share knowledge about the state of the art in toxicity assessment of aquatic habitats. The workshop consisted of a series of plenary discussions and task group working sessions. The first day was a plenary session. Experts gave presentations in each of four areas of toxicity assessment:

Population Risk Assessment;

Sediment Toxicity Assessment;

Whole Organism Toxicity Assessment;

Suborganismal Toxicity Assessment.

This initial plenary session involved the presentation of prepared papers which reviewed the relevant literature in each major topic and described the relationship between management needs for information and the available methods to fill these needs.

During days two and three, a selected group of experts remained to begin synthesizing information from the initial plenary session. Questions developed by workshop committees were used to provide structure, continuity, and commonality among each of the task groups. Each group, with the exception of the suborganismal, was charged with developing its own independent section to include conclusions, findings, and recommendations on the topic for that session.

The morning of the third day was used as a wrap-up session where task group members, within tasks groups, engaged in a review and a consensus-building process on the preceding day's products. The process provided a mechanism whereby discussion and identification of potential problems and limitations of the research procedures could be resolved. The final plenary session was used to present findings and make recommendations on how these findings could be applied to a pilot project.

This workshop resulted in general agreement on recommendations for a list of suitable methodolo-

gies and species that are appropriate for bioassessment.

Summary of the Opening Plenary Session

The first session was entitled Population Risk Assessment. The presenters were Ian Hartwell (University of Maryland), Donald Rodier (USEPA, Office of Toxic Substances), and Glenn Suter (Oak Ridge National Laboratory). Hartwell provided a historical perspective on the use of risk assessment. He noted that risk assessment originated in the insurance and human health industries, and has only begun to be applied to ecological and population assessments. As an environmental aid, risk assessment calculates risk to populations based on habitat, toxicant chemistry, and the organism's degree of exposure to a potential contaminant.

The second session of the opening plenary, Sediment Toxicity Assessment, worked towards better defining the complex relationships involving sediments and contaminants in the aquatic environment. John Scott (Science Applications International Corporation) and Chris Zarba (USEPA) presented the current state of knowledge regarding sediment toxicity testing. Scott particularly emphasized that sediment toxicity assays are important because sediments can be long-term reservoirs of dissolved and particulate-sorbed contaminants.

Methodologies for Whole Organism Toxicity Testing was the title of the third session. Steve Schimmel (USEPA) and Jeffrey Black (University of Kentucky) provided an overview of whole organism testing from both a laboratory and a field testing perspective. This session evaluated the most accurate, replicable, and cost-effective methodologies immediately available for analyzing toxic impacts on biota.

The fourth session, Methodologies for Suborganismal Toxicity Testing, began with D. G. Roesijadi (University of Maryland) presenting "Rationale for and Relevance of Analysis of Sub-organismal Responses to Contaminant Exposure." He suggested that the effects of toxicity within an aquatic system depend on both biotic and abiotic characteristics and processes that undergo complex interactions.

Roesijadi also pointed out that suborganismal responses are generally cellular and subcellular responses. He stated that one of the difficulties in the study of suborganismal responses is the problem of relating information derived at lower levels of biological organization to more complex levels; in other words, the ability to extrapolate effects from lower to higher levels of organization. Jay Gooch (University of Maryland) emphasized that the purpose of a suborganismal test is different from one testing the whole organism. He pointed out that one of the original justifications for the use of sublethal endpoints in environmental testing was the desire to have an early warning system. He went on to describe how suborganismal testing can be used to detect more subtle effects than traditional endpoints such as death. The relative merits of these two different approaches were discussed throughout the workshop.

Summary of Final Plenary Session

The final plenary session addressed the following concerns:

- identification of acceptable existing methods for risk assessment, whole organism testing, and sediment toxicity testing;
- suggestions for modification of existing protocols; and
- analytical discussion of the species and protocols identified.

The participants further distinguished between developed and developing bioassays. The former class is dominated by acute lethality tests; the latter class is

dominated by chronic and sublethal effects, some of which have not yet been field-verified. They also noted that laboratory studies can establish causal relationships, but their applicability to the field must be established. Each recommended bioassay was considered using the following factors: 1) biological responses; 2) technology available; 3) cost; 4) sensitivity to sublethal toxicity; and 5) significant effects.

Population Risk Assessment Group

The risk assessment group recognized the need for a quantitative approach. However, they indicated that initially the approach would need to be qualitative until a sufficient database was developed to allow a more quantitative assessment. This group recommended a mechanism that would allow researchers to characterize site-specific results in a ranking scheme to contrast different sites (Table 1). The ranking scheme will require additional work to evaluate precisely how to determine each ranking factor; however, the group did assign weights to each factor according to a consensus on its importance in evaluating toxicity.

Table 1. Risk Assessment Ranking System

Ranking Factor	Weight
Consistency of results	5.8
Severity of endpoints	8.5
Degree of response	7.9
Number of tests	4.8
Reproducibility of results	6.6

Note: Each member of the group subjectively assigned a score (1-10) to each factor to reflect its importance. The ranks were then averaged to provide an overall indicator of the group's opinion.

They concluded the following:

- Tests employing more than one species yield more information but require sensitivity calibration.
- Field effects (such as population depressions) may be due to direct toxicity or indirect community effects.

This group also made the following recommendations:

- Create databases of the relative sensitivities of assay species.
- Conduct specific cause-and-effect chemical studies.
- Estimate chemical exposure either directly or from historical data if the site is known.
- Define receptors and endpoints after an assessment of field biomonitoring, sublethal responses, and/or surrogate species.
- Design research to address a well defined gradient and a timefactor.
- Develop modeling to link field toxicity bioassays to population impacts.
- Address population parameters using endpoints from biomonitoring tests.

Sediment Toxicity Assessment Group

Participants agreed that sediment quality criteria (SQC) are needed. Because a large database would be required in order to develop SQC, the group emphasized the need to collect data supporting toxicity testing and chemical analysis. Recommendations from the sediment toxicity group were limited to tests that could identify hot spots and examine the condition of the sediment as a result of source inputs. In light of this, they placed strong emphasis on available methods that would include: (1) the 10-day amphipod test currently conducted in Puget Sound; and (2) basic screening tests that have been fairly cost-effective in Great Lakes (Table 2).

BIOASSAYS FOR SEDIMENT TOXICITY TESTING IN FRESHWATER, BRACKISH SALTWATER, AND HIGH-SALINITY ENVIRONMENTS IN THE CHESAPEAKE BAY

Organism	1 Availability	2 Taxonomic/geographic relationship to site inhabitants	3 Toxicological database	4 Demonstrated sensitivity	5 Ease of laboratory maintenance	6 Ease of test method	7 Ecological/economic relevance	8 Insensitivity to geophysical factors	9 Level of sediment exposure	10 Route of exposure	11	12 Test endpoints	Notes
Standard bioassays													
Freshwater													
Hyallela	X	X		X	X	X	X	X		So		L	
Chironomus	X	X	X							So		L	
Hexagenia		X	X	X		X	X		X			L	
Brackish saltwater (0-15 ppt)													
Hyallela										So		L	
Eohaustorius		?			X	X	X	X	X	So		L	
High salinity													
Rhepoxinius	X		X	X	X	X	X		X	So	a,b	L	
Ampelisca	X	X	X	X	X	X	X		X	So	a,b	L	
Bivalve larvae 48-hr pediveliger oyster *Mercenaria*	X	X	X	X	X	X	X			SS So	a,c a,d	 L	
Polychaete *Neanthes (Nereis)*		X	X		X	X		X	X	So		M	
Mysid (grass shrimp)	X	X	X		X	X	X	X	X			M	
Bioassays available based on enhancement of standard techniques													
Low salinity													
Leptocheirus	X	X		?	X	X	X	X	X	So		M	
Eohaustorius										So		M	
High salinity													
Lepidactylus	X	X	?	?	X	X	X	X	X	So		M	
Bioassay techniques under development or recommended for development													
Chronic tests													
Leptocheirus										So		H	
Lepidactylus										So		H	
Sago pondweed	X	X			X	X	X		X	So		H	

(10) So=solid phase; SS=sediment slurry

(11) a=acute lethal; b=behavioral effect; c=abnormal development; d=metamorphic failure, etc.

(12) L=low cost; M=moderate cost; H=high cost.

Table 2. Bioassays for sediment toxicity testing in freshwater, brackish saltwater, and high-salinity environments in the Chesapeake Bay.

Participants suggested the use of biological tests which use infaunal species to examine benthic community structure.

The group made the following recommendations:

> Use a multifaceted approach to choose the tests and organisms.
>
> Demonstrate the utility of an amphipod test for the Chesapeake Bay using existing standard methods and species indigenous to the Chesapeake Bay.
>
> Develop and field-validate tests for chronic and population effects of toxic sediments using Chesapeake Bay indigenous species.

Whole Organism Toxicity Assessment Group

This group took as its primary charge the question of what were the most appropriate laboratory and field toxicity tests for evaluating ambient toxicity in Chesapeake Bay. To address this issue the group developed a table of established and accepted methods, which considered species, duration, and type of test to be used (Table 3). Several species were recommended for evaluating ambient toxicity.

The participants emphasized that the choice of test organisms is contingent upon whether the proposed study has regulatory endpoints. The group suggested that species selection should encompass: 1) species pertinent for regulatory purposes; and 2) species not pertinent for regulatory purposes. Additionally, they agreed that no single test was adequate. Instead, they considered a suite of tests necessary to characterize toxic effects. Participants strongly recommended those testing methods that are being used as part of the Maryland and Virginia NPDES permitting process. They also recognized the need to use ecological indicator species and species amenable to laboratory testing.

Final Plenary Session Recommendations

Participants in all three sessions of the Ambient Toxicity Assessment Workshop reached the same technical consensus on the following issues. These include:

> Toxicity tests which measure growth and reproduction, as well as survival, are highly desirable.
>
> Acute lethality tests for Bay species are not sensitive indicators of ambient toxicity and should not be used routinely. However, in areas where high toxicity is suspected, acute lethality testing may be used as an initial screen.
>
> Chronic or partially chronic tests with sublethal endpoints are the preferred methods.
>
> Test categories were established that included: 1) established regulatory methods; 2) established Chesapeake Bay specific methods; 3) Chesapeake Bay research methods still being developed.
>
> Toxicity assessments should recognize the following major salinity regimes: 1) freshwater (0 ppt salinity); 2) estuarine (> 0 to 20 ppt); 3) marine (> 20 ppt).

In summary, information generated by this workshop will offer resource managers a technical appraisal of the strengths and weaknesses of methods potentially available to develop final guidance for a pilot study of ambient toxicity in Chesapeake Bay. Findings and conclusions from this pilot study will be used to develop a Basinwide ambient toxics monitoring program.

Table 3· Whole organism tests for ambient toxicity in freshwater, estuarine, and marine environments of the Chesapeake Bay.

Category	Freshwater environment	Estuarine environment (max. 25 ppt)	Marine environment
Proven Regulatory Methods	*Ceriodaphnia* 7-day chronic Fathead minnow 7-day chronic and embryo/larval test *Selenastrum* 96-hr	Sheepshead minnow 7-day chronic and embryo/larval test *Menidia beryllina* 7-day chronic *Mysidopsis bahia* 7-day chronic Bivalve larvae: *Crassostrea virginica* *Mya arenaria* *Mercenaria mercenaria* *Skeletonema* algal test 48 hr?	Sea urchin fertilization test *Menidia beryllina* 7 -day chronic Sheepshead minnow 7-day chronic *Mysidopsis bahia* 7-day chronic Bivalve larvae test: *Mytilus edulis* *Champia parvula* reproductive
Established Methods Pertinent to Chesapeake Bay	Embryo larval: bluegill, catfish Striped bass larval toxicity test	Grass shrimp larval acute lethality, 96 hr Striped bass larval toxicity test (low salinity)	Grass shrimp larval acute lethality, 96 hr Striped bass juvenile 96-hr acute lethality
Research Methods Pertinent to Chesapeake Bay	Duckweed 96-hr	*Anchoa mitchilli* 96-hr test *Callaglossa* algal chronic Sago pondweed toxicity test *Eurytemora affinis* *Neomysis americana* 96-hr acute *Acartia tonsa*	*Acartia tonsa* 7-9 days *Anchoa mitchilli* 96-hr acute lethality (larval)

INTRODUCTION

This document contains the proceedings from the Ambient Toxicity Assessment Workshop which was held in Annapolis, Maryland on July 25-27, 1989. Funding for the Ambient Toxicity Assessment Workshop was provided by the Coastal Zone Management Act of 1972 (as amended), Section 309 (Interstate Grants) as administered by the Office of Ocean and Coastal Resource Management, National Oceanic and Atmospheric Administration. State logistical and planning support was provided by the Section 309 Planning Work Group, Maryland Department of Natural Resources, Virginia Water Control Board, USEPA Chesapeake Bay Liaison Office, and the Chesapeake Bay Program Scientific and Technical Advisory Committee.

The workshop was held in response to two commitments outlined in the Chesapeake Bay Basinwide Toxics Reduction Strategy (Chesapeake Executive Council, 1988). One commitment is:

"By July 1989, the signatories agree to convene a scientific workshop to develop consensus protocols for the use of biological indicators to monitor the effects of toxic contaminants in Chesapeake Bay habitats important to living resources."

The findings and conclusions from this Workshop are intended to provide guidance for the implementation of a related commitment:

"By December 1989, the signatories commit to develop and begin to implement a plan for Baywide assessment and monitoring of the effects of toxic substances, within natural habitats, on selected commercially, recreationally and ecologically important species of living resources."

The report is arranged as follows: first the report of the opening plenary session, followed by the report of the final summary session, then by Appendices containing (A) workgroup session reports, (B) list of participants, and (C) results of the Bioassay Capabilities Survey.

Plenary Session A:

Population Risk Assessments Based on Toxicity Testing

Convener: Ian Hartwell

Opening Remarks

I want to put forth a few general comments on what ecological risk assessment is and how it can be used before we hear more detailed discussions on numerical approaches and applications from the plenary speakers. The basic concepts and approaches to risk assessment were originally developed in the fields of insurance and human health for the purposes of predicting longevity and injury frequency. These ideas have been expanded and adapted to engineering, industrial operations, and other fields, and most recently have been applied to pollution assessments.

Fundamentally, a risk assessment is a calculation of the probability of some outcome, based upon the hazard of some input parameter and the degree of exposure to that parameter. The application of risk assessment to ecological situations is a relatively new field. As with any new field, there are a lot of specialized terms that mean specific things to those involved in the area of risk assessment, but that are often used interchangeably by those not working directly in that field. These inconsistent uses are a source of confusion. Thus I would like to review briefly some of the terminology and approaches used in risk assessment. It is important to realize that different kinds of assessments yield different products with specific uses.

"Hazard assessment" is a determination of the inherent danger of a given chemical substance or activity. It requires a measure of some sort of effects endpoint and a dose.

It is a quantification of the relationship between dose and response. It should include information on both dose concentration and duration of dose. How inherently dangerous is the material in question, without regard to potential exposure?

"Exposure assessment" is an estimation of concentrations of a chemical substance to which target organisms are exposed. It includes estimates or measures of quantity of material, fate and transport modeling, transformations, and bioconcentration potential. The estimates are made without regard to the hazard of the material. Where does it go, and in what concentration? What are the pathways to the target organisms, and how long will they be exposed?

"Receptor characterizations" address the entity of interest in the risk assessment that may be affected by exposure to chemical substances. In our context, this may include a species, a population, a community, or an ecosystem. We need to be able to define what the receptors are and what other important parameters affect exposure or response, such as sensitive life stage, migratory patterns, feeding habits, etc.

"Risk assessment" is the integration of the hazard assessment, the receptor characterization, and the exposure assessment. It estimates the magnitude and probability of harm resulting from exposure of the target organisms to a hazardous substance or activity. This term includes all of the other three and is thus more complex, and it requires that the other three be compatible.

There are some other items used in combination with these terms that are relevant here. These are "endangerment assessments" and "damage assessments."

"Endangerment assessments" are used in the Superfund process for waste site remediation. They are basically a preliminary, hypothetical risk assessment for a National Priority List site or a proposed site to

document whether there exists current or potential risk of significant exposure to toxic chemicals. They examine the presence of chemicals for dangerous concentrations, potentially exposed populations, and the presence of exposure pathways. That is as far as they go; they do not make predictive conclusions beyond either "Yes, there is potential risk," or "No, there is not."

"Damage assessments" are field-based evaluations of environmental damage after the fact. They were designed for spill sites. That is, after a release of toxic chemicals in harmful quantity, the damage assessment is a means of quantifying the environmental harm and (in current practice) arriving at a monetary cost for reparations and/or cleanup. That is not to say that damage assessment procedures could not be used as the basis for input into a full-blown risk assessment.

The approaches in generating a risk assessment can be grouped into qualitative and quantitative methods. In part, the method of approach is governed by the proposed uses of the assessment. You can use them to help set priorities, for example, for disposal options, for site selection, or for research directions. You can use them to set standards. You can use them as input into risk management at the regulatory level or for resource protection or restoration.

Qualitative methods are obviously useful when you have little or no data. They rely heavily on professional judgment and can be used for setting priorities as ranking or screening procedures. They are not useful for standards development or risk management. They don't yield quantitative estimates of magnitude or probability of effect.

Quantitative methods can be broadly divided into quotient methods and exposure-response methods. Quotient methods basically compare some predicted effect level with a benchmark standard. This requires that you have a standard against which to compare your environmental concern level. The concern level can be a toxic concentration, an acceptable level of population reduction, or some other measure of environmental effect, which may or may not be an extrapolated value. Quotient methods give you a yes/no prediction, or in some methods a yes/no/maybe prediction. They do not give you a probabilistic result, only an indication whether you are above or below some benchmark.

Exposure-response methods are modeling approaches and can be subdivided into top-down or bottom-up methods. These methods require vastly larger data sets and knowledge of the relevant environmental system than other approaches. However, they have the potential to provide both a prediction of magnitude and probability of effect.

Top-down models require ecosystem response data as input (that is, field data). They evaluate impacts on the ecosystem directly. They don't provide a mechanistic reason for the changes that are seen or predicted. The models don't need to and cannot determine the structural or functional changes occurring in the environment as a result of chemical or physical effects.

Bottom-up methods require laboratory toxicity data and a great deal of site-specific and chemical-specific data. They can model community-level effects, and through the model development process they can provide information on how the system works. At present we still don't have the tools to incorporate all of the aspects of life history, fate and transport of chemicals, or stochastic variations in the ecosystem into unified models, particularly for a system as large as Chesapeake Bay. However, great progress has been made over the last several years and new methods of population compensation modeling are being developed.

All quantitative methods must also have some means of estimating uncertainty in parameter estimation. We

need data on confidence intervals for all our input data and our extrapolations. This can often be one of the most difficult aspects of risk assessment. How confident are we that this result will protect the environment -- a factor of two, an order of magnitude, three orders of magnitude? This has to be calculated or estimated. And there are a variety of approaches such as sensitivity analysis, calibration, or validation, depending upon what type of risk assessment approach you are using.

Finally I want to say a few words on the difficulty of applying to the environment a mathematical procedure whose basic concepts were developed for human health predictions. We need to be mindful that some people who will be using the results of our efforts do not always understand that the databases like those available for risk assessment in human health simply do not exist for any other species in such detail. As Dick Tucker, formerly with the EPA, used to say, we know how much exposure to benzene will cause chronic headaches in people, but we will never know how much it takes to produce the same effect in ducks. We don't have the databases to achieve the same precision. There are thousands of species to be considered, with thousands of interactions and life history details which we do not know, and there are myriads of other influences such as habitat integrity, environmental resilience, and normal ecological variability which increase our uncertainty. It is possible to do ecological risk assessments, but the inputs and the nature of the target populations dictate that the approach and the results may not be as clean-cut as health assessments.

This may sound like a pessimistic introduction to the prospects of ecological risk assessment, but it's important to remember we are dealing with vastly more complex systems than risk assessments have addressed before. Risk assessments are currently being used successfully in the regulation of pesticides, effluents, new industrial chemicals, and air pollution, and they are under development for use at Superfund sites. Risk assessment provides a scientific basis for decision-making, for resource allocation, and for answering questions which concern the regulatory community. For those reasons, it has great potential for improving our ability to communicate scientific concerns that are both politically and legally defensible to the public, industry, and resource managers.

Assessing Risks to Populations

Donald J. Rodier

Introduction

Good morning and thank you for inviting me to your workshop. I am a biologist in the Environmental Effects Branch, which is in the Health and Environmental Review Division of the Office of Toxic Substances. We are a support branch of the US EPA and are responsible for providing ecological hazard and risk assessments of industrial chemicals. Because these chemicals are commonly discharged to bodies of water, we have concentrated our efforts on developing methods for evaluating both the hazard and risk of these chemicals to aquatic populations.

There are two main categories of industrial chemicals: 1) new chemicals and 2) existing chemicals. Existing chemicals are those on the Toxic Substances Control Act (TSCA) inventory (a list of chemicals manufactured before July 1, 1979) and in common use. Chemicals that are not on the TSCA inventory are considered new. The two types differ in regard to the amount of data and the time frames in which we have to act upon them.

Before proceeding further, it must be emphasized that while considerable progress has been made in the area of ecological risk assessment with regard to population and ecosystem models, such methods are still in the developmental stage and have not been used in regulating industrial chemicals. At this time, we are not aware of any one fully accepted method that will translate the data from laboratory assays to precise

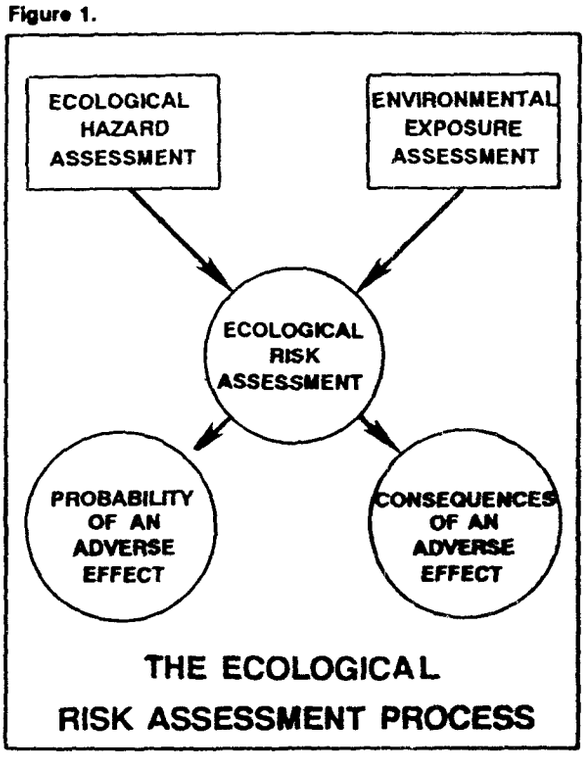
predictions of effects on natural aquatic populations.

Conceptual Framework of an Ecological Risk Assessment (Figure 1)

Ecological risk assessment is a logical process whereby one integrates the results of the hazard and exposure assessments of a chemical or other stressor into a statement regarding the probability and consequences of an adverse ecological effect.

Unlike human health risk assessments, which have readily understood endpoints such as carcinogenicity, teratogenicity, etc., ecological risk assessments utilize endpoints obtained from toxicological assays. These include an algal EC10, a fish LC50, and Maximum Acceptable Toxicant Concentration (MATC). Frequently we are asked "What is the real significance if such endpoints are exceeded in the wild?" That is, what will happen?

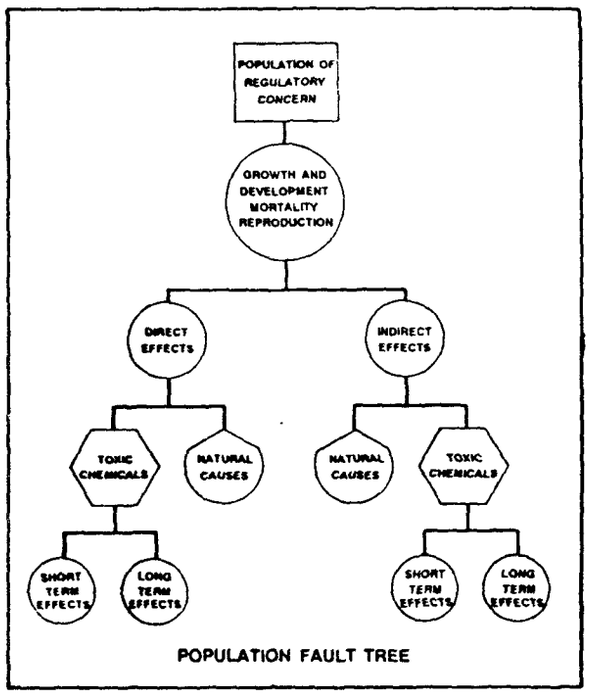
Figure 1.



In the Environmental Effects Branch, we relate the results of ecological risk assessments to "Populations of Regulatory Concern." This term was arrived at after a review of 268 environmental legislative acts. In summary, all past and present environmental legislation was enacted to protect natural resources (both biotic and abiotic) which were valued by society, from any reduction, degradation, or loss in any quality, quantity, or utility (Clements, 1983). After reviewing nine incidents of how chemicals affected natural biotic resources, we concluded that chemicals caused adverse effects on growth and development, mortality, and reproduction, and that such effects were manifested at the population level of organization.

Putting all of this together (Figure 2), we see that the focus of an ecological risk assessment is on natural populations that are valued by society for various reasons (aesthetic, commercial, ecological) or are already protected under different statutes such as the Endangered Species Protection Act.

Figure 2.



The three factors that govern a population are growth and development, mortality, and reproduction. If individuals fail to grow and develop, the population may be in danger of not surviving. In addition, if the population is a commercially valuable one, the utility of that population is likely to be reduced. If there is mortality above the normal death rate, the population may also be in danger. And if individuals within the population fail to reproduce, the population is obviously in danger of extinction.

Toxic chemicals can adversely affect mortality, growth and development, and reproduction in two ways: direct and indirect. Direct effects are simply toxic effects with direct impact on growth and development, mortality, and reproduction of a specific species or population. In evaluating the hazard of a particular substance, we evaluate the direct toxic effects of a substance to various surrogate species.

Indirect effects are more difficult to define, interpret, and measure. Indirect effects include disruptions in the habitat of a particular population and alterations in the food supply. Natural populations do not exist in a vacuum. The well-being of one population is dependent upon other populations which either act as a food source for that population or play a role in governing the growth of that population through predation or grazing. Toxic chemicals can alter these functions.

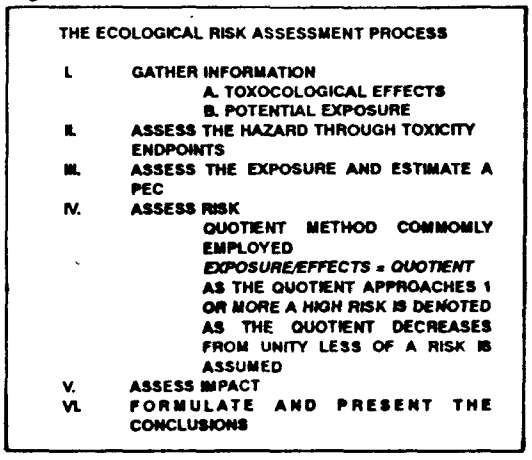
In addition to the effects of a toxicant, we know that unperturbed populations are influenced by their natural environment, and the term "natural causes" is a catch-all to include the natural variability associated with population maintenance. In addressing the risks posed by toxic chemicals, both the short- and long-term effects must be considered. Such evaluations are obtained from the hazard assessment.

I want to emphasize that Figure 2 presents the conceptual framework of an ideal ecological risk assessment -- that is, the ability to quantitatively address the risks of direct and indirect toxic effects to specific populations of regulatory concern. We are not there yet but we are making progress. As of right now, we can only make inferences to effects on such populations.

How Ecological Risk Assessments Are Conducted in EEB (Figure 3)

Existing chemicals. Chemicals are referred to EPA by an Inter-Agency Testing Committee for testing. We have one year to respond. Testing proceeds according to procedures we have set forth (USEPA, 1983). The tests utilize surrogate species (USEPA, 1982) and published guidelines (USEPA, 1985).

Figure 3.



New chemicals. New chemicals, or PMNs (premanufacture notice submissions), present certain problems. The major problems are the large number submitted (over 1000 annually), the short turn-around time (90 days allowed for evaluation), and the scarcity of data (USEPA, 1986).

Because there is little data to evaluate the ecological hazards of a PMN, EEB utilizes structure activity relationships (SARs) to estimate potential toxicity (Auer et al. 1989). SAR includes an evaluation of a chemical by comparison to another chemical for which there is data (analog) and also through regression analyses of specific chemical classes.

A large number of these SARs have been compiled and are available (Clements, 1988). To predict the concentration of a particular PMN that is likely to cause some adverse effect in the environment, assessment factors (USEPA, 1984) are used (Figure 4). Assessment factors are numbers that account for the uncertainties due to variables such as test species sensitivity to acute and chronic toxicity, laboratory test conditions, and age group susceptibility.

Figure 4.

APPLICATION OF ASSESSMENT FACTORS TO EVALUATE NEED FOR TESTING

DATA AVAILABLE	ASSESSMENT FACTOR TO BE APPLIED
QSAR-CALCULATED LC50	1000X
SINGLE LC50 FOR ANALOG	1000X
TWO LC50's FOR PMN (e.g., 1 FISH, 1 INVERTEBRATE)	1000X
TWO LC50's FOR SAME ANALOG (e.g. 1 ALGAE, 1 FISH	1000X
THREE LC50's FOR PMN (FISH, ALGAE INVERTEBRATE	
THREE LC50's FOR SAME ANALOG (FISH, ALGAE, INVERTEBRATE)	100 X
FIVE LC50's FOR PMN (e.g. 2 FISH, 3 INVERTEBRATES)	100X
FIVE LC50's FOR SAME ANALOG (e.g. 3 ALGAE, 2 FISH)	100X
MATC FOR ANALOG	10X
MATC FOR PMN	DATA-BASED DECISION ON NEED FOR FURTHER TESTING OR REGULATORY DECISION (ASSESSMENT FACTOR NOT USED

Exposure assessments are conducted in the Exposure Evaluation Division. Models are used to predict the concentrations of a particular PMN in various stream reaches (percentiles). In the initial assessment, estimates are made of concentrations under mean and low flow conditions. Later, a concern level derived using assessment factors is used with the probabilistic dilution model (USEPA, 1988) in order to evaluate how often (i.e., how many days out of the year) that concern level will be exceeded.

The quotient method (Barnthouse et al., 1986) is used to compare the concern level or toxicological endpoint with the various exposure concentrations or with the probabilistic dilution model. This method has been used successfully both by the Office of Toxic Substances (Rodier, 1987) and the Office of Pesticide Programs (Urban and Cook, 1986). However, the following deficiencies associated with the quotient method have been noted:

- It does not routinely take into account dose responses, other than standard toxicological measurements such as an LC50 or MATC.
- It has no predictive capability. It does not address taxonomic or life-stage sensitivities to a toxic chemical.
- Indirect effects of a toxicant are not readily addressed.
- Implicit in the use of the quotient method is the assumption that the exposure duration will be as long as (or longer than) the toxicological test duration. Thus pulse or short-term exposures are not readily addressed.
- The uncertainties associated with extrapolations from laboratory data to natural environments are not easily addressed.

Refinements to the Quotient Method

Figure 5.

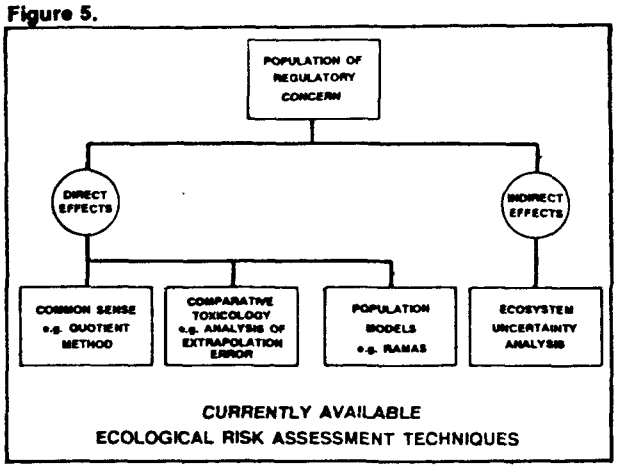


Figure 5 shows some of the extant ecological risk assessment methodologies. The quotient method has already been discussed. The author of the Extrapolation of Error method, Dr. Glenn Suter, has previously discussed this methodology in depth. The Risk Analysis and Management Alternatives System (RAMAS) was originally designed for the Electric Power Research Institute (Rohlf et al., 1986). RAMAS is a user-friendly Monte Carlo Simulator of age-structured populations. It is designed to answer the following: 1) What is the probability that a population size will fall below a given threshold within a specified time, and 2) what are the number of individuals in a certain age class within a specified time? EEB currently has a rainbow trout version of RAMAS which is still being evaluated. Inputs to population models include maximum age of a given population, fecundity, survivorship among the various age classes, and estimates of toxicant-induced mortality among the age classes.

The Ecosystem Uncertainty Analysis (EUA) was developed by O'Neill et al., (1982) at the Oak Ridge National Laboratory as part of the Office of Research and Development Synfuel Program. Believing that the EUA held some promise in addressing indirect toxic effects, we sponsored work on additional refinements (Bartell, 1987, 1987a). Time does not permit discussion of all aspects of EUA, but I would like to briefly explain the concept and then show how it can be used to address the "Consequences" part of an ecological risk assessment.The model used in EUA is the Standard Water Column Model (SWACOM). SWACOM mimics the aquatic populations that occur in the water column. There are four groups of populations represented: (1) phytoplankton or algae whose biological role is the fixation of energy through photosynthesis, (2) zooplankton or aquatic invertebrates that consume phytoplankton and serve as a food source for (3) the forage fish, which in turn are fed upon by (4) the top carnivore or predator fish. The top predator fish can be thought of as a population of regulatory concern.

Figure 6.

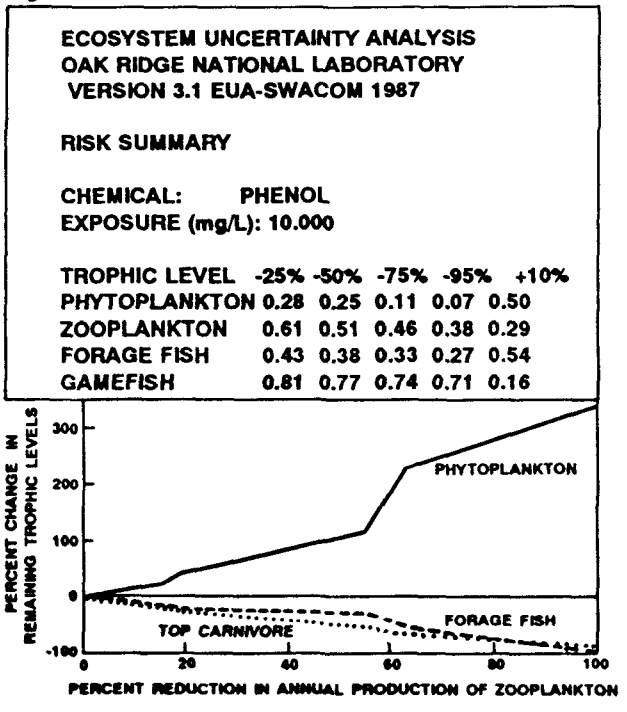


Figure 6 shows what could happen if a chemical which was highly toxic to zooplankton entered a lake. Five simulations with SWACOM were performed (Bartell et al., 1987). Each simulation decreases the zooplankton in 20% increments and represents a one-year cycle in a lake. The decrease in zooplankton causes a decrease in the forage fish population. The

top carnivore population decreases because of the declining forage fish population. Because the phytoplankton are no longer grazed by the zooplankton, they begin to increase. This can lead to so-called algal blooms, which are undesirable not only because they discolor bodies of water and create noxious odors but also because they decrease the oxygen content of the water, causing fish kills. This example demonstrates the importance of indirect effects, and the model simulations aid greatly in both evaluating and explaining such effects.

Since 1986, The Office of Research and Development has been supporting an ecological risk assessment initiative for the Office of Pesticides and Toxic Substances (OPTS). Some of the products developed thus far are the following:

- Food and Gill Exchange of Toxic Substances (FGETS) Model for estimating uptake of neutral hydrophobic chemicals in fish.
- Approaches to assessing effects of neutral hydrophobic chemicals on aquatic chemicals.
- Aquatic Plant Uptake Model.
- Center for Exposure Assessment Modeling (CEAM).

Summary

In summary, I offer the following considerations:

- There is a legal and scientific basis for using populations as the unit of ecological risk assessment.
- The quotient method of ecological risk assessment is used in the Office of Pesticides Programs and the Office of Toxic Substances. In spite of its deficiencies, it has been used successfully to regulate industrial chemicals and pesticides.

Population and ecosystem models are being employed in our office to evaluate the consequences of exceeding certain toxicological endpoints. As such, they are currently being used as adjuncts to the quotient method as opposed to replacing the quotient method.

Questions

Q: I had the impression that proposers of new chemicals must bring quantities of toxicity data for new chemicals. Is that incorrect?

A: Yes, that is incorrect.

Q: I had the impression that you go through a full analysis independent of that.

A: Yes, we do. Because of the paucity of data we rely on the QSAR's and SAR's, analogs, and a lot of databases to get those estimates. I might also add that in order to register a pesticide (as opposed to a new chemical) a great deal of test data is necessary.

Q: How much experience have you had with the verification of these population and ecosystem models; how confident are you in your predictions?

A: We did an evaluation of the SWACOM model with outdoor ponds. The models were picking up effects as we became more specific with the information we gave it. If we gave it analog data, it didn't respond. As we gave more precise data, the model predicted effects different from those observed in the ponds. Finally when we modified the model we got good effects -- the model predictions compared favorably to the actual pond's effects. Assessment factors worked

surprisingly well for the ponds. Note that this was only one study and we are continuing to compare model predictions with data obtained from field studies.

Q: What criteria are used to draw the line in the registration of a chemical for manufacture?

A: The first option is that testing be done by the manufacturer, both for human health and ecological effects. About 60% of the time they elect to do the testing and we take it to the point where either the concern levels aren't being exceeded, in the case of ecological assessment, or we're getting distinct "no effect" levels, in the case of human health.

Rodier further explained the last answer at a later date. He wrote: "Judging from the use of the term 'registration' there may be some confusion about my office and OPP (Office of Pesticide Programs). OPP registers pesticides; we [OTS] do not register industrial chemicals. The basic criterion for both OTS and OPP is that the chemical not cause unreasonable risks to human health and the environment. In the case of OTS, if a substance is deemed to pose a risk to human health or the environment, testing can be required. These tests proceed in a tiered fashion, going from short-term acute tests to more complex chronic tests. The outcome of the first set of tests determines whether additional testing is necessary. In addition to evaluating the hazard of a particular chemical, the potential exposure of that chemical to humans or the environment is evaluated. If the risk assessment indicates no unreasonable risks, the chemical is allowed to be manufactured."

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Population Risk Assessments Based on Toxicity Testing

Glenn W. Suter II and L. W. Barnthouse (presented by Dr. Suter)

Defining risk assessment

Risk assessment estimates the probability of undesired events. In practice, risk assessors attempt to quantitatively define the relationship between the assessment endpoint and the available information concerning the particular environment, the environmental chemistry of the pollutant, and the toxicity of the pollutant. Examples of relationships that must be inferred include those between measured environments and the assessed environments, between the environmental chemistry of a chemical and its concentration in various media (i.e., transport and fate models), and between toxicity test endpoints and assessment endpoints.

Risk assessments quantify uncertainty in the data and in modeling assumptions and estimate their contribution to the uncertainty concerning the effects of the pollution (i.e., the probability of having effects that exceed the assessment endpoint). There are two types of risk assessment. Prospective (or predictive) risk assessment is useful in finding the potential effects of new chemicals before they are released into the environment. Retrospective risk assessment is concerned with the continuing effects of events that began in the past. Most of the concerns in the Chesapeake Bay involve retrospective risk assessment.

For a predictive assessment, the first step is to select endpoints of concern. These must be well-defined, as, for example, striped bass abundance or oyster production. Then the source terms, estimates of the rate at which a chemical or chemical mixture is released, are developed. The type of environment that is of concern must be determined. Then an effects assessment and an exposure assessment must be done for that source term and environment. Finally, the exposure assessment and effects assessment are combined for an estimate of risk.

Use of whole organism toxicity data

Standard organism-level toxicity tests and endpoints exist, but they are not designed for risk assessment. These tests are not applicable to the very short exposures (i.e., spills) or longer chronic exposures that interest us. Endpoints for these tests are calculated using hypothesis testing statistics, which are inappropriate for risk assessment. In addition, sensitive and important responses may not be measured, temporal dynamics may be neglected, and results may be inadequately reported.

Test endpoints that reflect population properties can be calculated. Examples include (1) weight of young per female, (2) intrinsic rate of increase (r), and (3) reproductive potential. In addition, tests can be designed to tell you about the temporal dynamics of effects. This information can then be used in population or ecosystem models to give a good estimate of effects.

Use of whole-organism toxicity in predictive assessments. Conventional test endpoints can be used in the quotient method to rank chemicals. In this standard method, the quotient calculated is the test endpoint/-estimated environmental concentration.

With use of the Analysis of Extrapolation Error method, the probability of exceeding toxicological endpoints can be extrapolated in several ways. Endpoint values can be extrapolated for untested species; the more closely related organisms yield more similar responses. Extrapolations can be made for different life stages of the same organism. Temporal extrapolations under different exposure dynamics can give information on concentration/duration/response relationships.

Populations of interest can be modeled and toxic effects that are parameters of the models (mortality, fecundity, growth) can be incorporated. The output of the model is the probability of a particular level of effect on a population attribute (e.g., probability of a reduction in numbers of adult fish > 0.1). Four dimensions can be addressed: concentration, duration, severity of response, and proportion of the population responding. The goal is to produce a response surface incorporating severity of effect, concentration, and duration; this response surface is the basis for predictions.

For population models, toxic effects are measured by parameters such as mortality, growth and fecundity. Extrapolation analysis is used to estimate parameters of the population model from the toxicity data. Extrapolations from toxicity data to model parameters are a greater source of variance in fish population models than differences in life history or fishing pressure.

Examples of extrapolations:

- LC50s of Perciformes can be used to predict LC50s for Salmoniformes.
- An MATC (maximum allowable toxicant concentration) can be predicted from an LC50, but there is a lot of scatter because the MATC is not a consistent endpoint.
- An LC50 can also be used to predict a specific chronic effect such as an EC25 of egg hatchability; this is a much better prediction than the MATC.

An estimate of the risk of a parameter such as MATC being exceeded in the field can be determined by the overlap of the contaminant concentration in the field and the MATC distribution predicted by the extrapolation methods.

By looking at the fisheries data models, behavior of the model can be statistically matched to time series. Population models can then be used to look at the effects that differences in life history among fishes have on the influence of toxicity.

Ideally, we would like to do full life-cycle tests for organisms of interest. But in lieu of this, the percentage reduction in abundance as a function of concentration can be determined by the model. This has confidence bounds which can be quite tight if the data are good. But if you only have an LC50 for a surrogate species, the confidence bounds can be up to two orders of magnitude.

Use in retrospective assessments -- assessments of past and ongoing pollution. Population and whole-organism responses in the field can be used to establish the nature, magnitude, and association with pollutants of the assessment endpoints. Another approach is to use whole-organism responses in toxicity tests to establish that the pollutant exposure levels in the field could cause the effects observed in the field. If the species, life stages, etc. in the laboratory are different from those in the field, extrapolation models must be used. Population and ecosystem models are also used to match laboratory responses of individual organisms to population-level effects in the field.

Use of suborganismal toxicity data

No consistent endpoints have been developed for these assays, and they are not regularly used in assessment. For use in predictive assessment, suborganismal responses are generally more sensitive than conventional organismal responses, but their use to predict effects on populations is problematic. One reason is that their inherent importance is not clear -- nobody cares about a fish's histology or enzyme levels per se. Also they have not generally been shown to be correlated with responses that are important; in many cases, the correlation has not been investigated. A third difficulty is that there are no

models to extrapolate suborganismal responses between taxa, life stages, and exposure durations.

In retrospective assessment, suborganismal toxicity data can be of use in two ways. First, it can supplement organismal and population responses by serving as the basis for a diagnostic syndrome. It can provide evidence of the regularity of association of a pollutant and an effect -- evidence for which organismal and population responses are too generic. It may also provide a link between field observations and toxicity tests. (If symptoms are the same then they provide evidence of common causation.)

Suborganismal toxicity data can also be useful if the population of interest is no longer present. In this case the suborganismal responses of more resistant species could provide evidence that the loss was due to pollutant effects. This use again raises the issue of correlation of suborganismal with organismal and higher effects: do the suborganismal responses lead to organismal responses? It also raises the issue of extrapolation again: is it credible that a more sensitive species would become extinct at a pollutant exposure level that caused the observed suborganismal responses in the presumed resistant species? The answers to these questions must come from toxicity testing. One cannot expect to observe a population as it is undergoing a pollution-induced crash and measure suborganismal responses.

Use of sediment toxicity data

In theory, organism-level data in sediment would be treated like organism-level data in water, and suborganismal data would also be treated the same in all media. In practice, only the quotient method and similar nonpredictive approaches have been used with sediment toxicity data. This is partly because the problem of exposure assessment has not been resolved. Likewise, appropriate assessment endpoints have not been established. For instance, are we concerned about inherently valuable benthic species? Are we concerned about food species for fish? This matter of defining endpoints is a regulatory and political concern as much as a scientific one. We must remember that what we are working for in risk assessment is a regulatory tool.

Questions

Q: Have you used additivity models to predict interactions between multiple components?

A: Yes, we use concentration additivity models because of Lloyd's evidence that the most common mode of chemical interaction is additivity and that synergisms are extremely rare or nonexistent.

Q: How do you calculate probability when you extrapolate more than once; for example, when you go from LC50s of one species to some other measure of toxicity?

A: There are two ways to do that. First, one can directly regress the chronic response, the *MATC for the species you are interested in,* against the LC50 for the test species and skip all the intermediate stages. Use the initial X and the final Y. Second, one can perform the extrapolation in multiple steps and carry the variance from one step to the next.

Q: But how do you determine the error you are losing at each step?

A: Well, you are not losing anything the first way because you are directly regressing the endpoint response against the measured response. For the second method, you can add the variances of each of the regression steps.

Q: Are you saying that suborganism level programs are not useful because you cannot connect with populations or are you just saying that these experiments haven't been done?

A: The latter. I hope they'll be done, in order to allow us to make these extrapolations.

Q: But don't you need that kind of information to really understand the population?

A: Yes, but you don't know whether the animal is actually going downhill, or if it is just adapting and it will continue to live and reproduce. This will be valuable if it can be shown to have effects on the organisms as a whole.

Q: Are parameters other than reproduction, growth, and mortality such as those that affect recruitment being taken into account?

A: Recruitment is a function of reproduction, growth, and mortality. The example you mentioned, predation on the larvae of intertidal invertebrates as they pass through kelp forests, is simply a specific ecosystem-level mechanism influencing one of the population parameters. To explicitly incorporate predation, competition, etc., you would need an ecosystem model. These processes are implicit in the population model's background survivorship.

Comment from audience: The bottom line is really the number of organisms that enter the population.

Q: What is an acceptable endpoint for risk assessment in terms of population impact? Is it a 5% reduction in community structure? Until that is determined, modeling processes have very little meaning.

A: Identification of endpoints is more a regulatory and political process than a scientific one. The political process will decide this.

Plenary Session B:

Sediment Toxicity Assays

Convener: Ray Alden

Considerations for Sediment Toxicity Tests

John Scott

Introduction

The questions and issues pertaining to sediment toxicity assays that this workshop has been asked to address are:

1) What routes of exposure of contaminants should be assessed?

2) What toxicity test methods are currently available?

3) How can these methods be applied to the Chesapeake Bay system and what is the feasibility of these methods for large-scale monitoring?

4) What species should be selected for these assays and what should the selection criteria be?

5) What biological endpoints should be selected that would be appropriate?

6) Can one extrapolate among responses, i.e., from suborganismal to acute and chronic responses to resources at risk?

7) Sample collection and experimental design issues will be addressed at the workgroup sessions.

8) How do sediment toxicity test results apply to risk characterization?

Toxicity tests are quantitative measures of contaminant impacts on survival, behavior, genetic processes, physiology, reproduction, and other biological processes of higher order. They are used to assess media-specific toxic effects and can also provide an integrated response to complex mixtures.

The responses most commonly and historically used have included lethality and generation of LC50s.

More recently methods have been developed to examine sub- and supra-organismal responses.

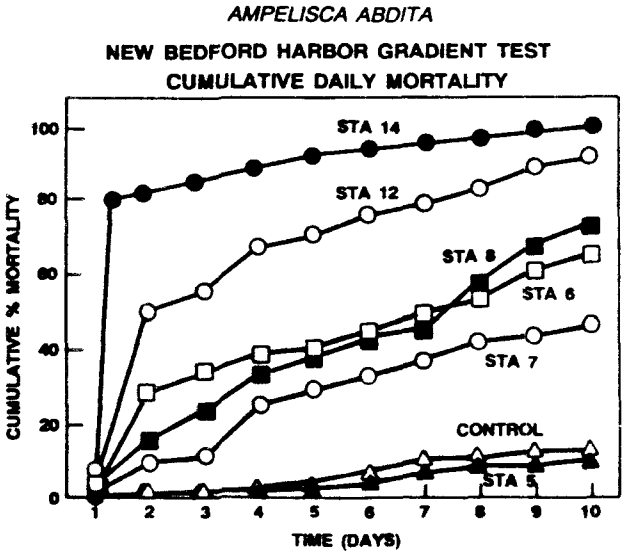
Sediments are important because they can act as long-term reservoirs of dissolved and particulate-associated contaminants, and benthic organisms are a major vector in food chain transfer and biomagnification. There are many factors controlling contaminant availability and, hence, toxicity in natural systems. There is biological mediation of contaminant flux via biodeposition, bioturbation, and bioaccumulation. More information is needed to understand sediment contaminant availability and how it is influenced by factors such as organic carbon content, grain size, water content, and oxidation state.

Sediment toxicity tests have had many applications. The primary impetus for sediment testing has been through the regulatory process, specifically for dredge-material permitting, where a recommended suite of tests were put forth by the EPA and the Corps of Engineers in the 1977 "Green Book." These tests commonly used a burrowing clam, a

burrowing polychaete, and epibenthic shrimp or fish for 10-day exposures, and then quantified survival and bioaccumulation. Sediment toxicity tests have been used widely on the West Coast (e.g., Puget Sound, San Francisco Bay) to monitor spatial and temporal trends of contaminant effects in sediments. A subset of this type of monitoring deals specifically with remedial action studies at Superfund sites, identifying hot spots and looking at the condition of sediments as a result of source inputs. Finally, sediment toxicity tests are used in the research arena to examine modes of toxicity and contaminant-specific exposures that result in tissue-specific pathologies or physiological responses. They are being used to define extrapolation potential across the biological hierarchy, from sub- to whole- to supra-organismal responses. These methods are also being used to evaluate contaminant bioavailability through tests with spiked sediments and field sediments.

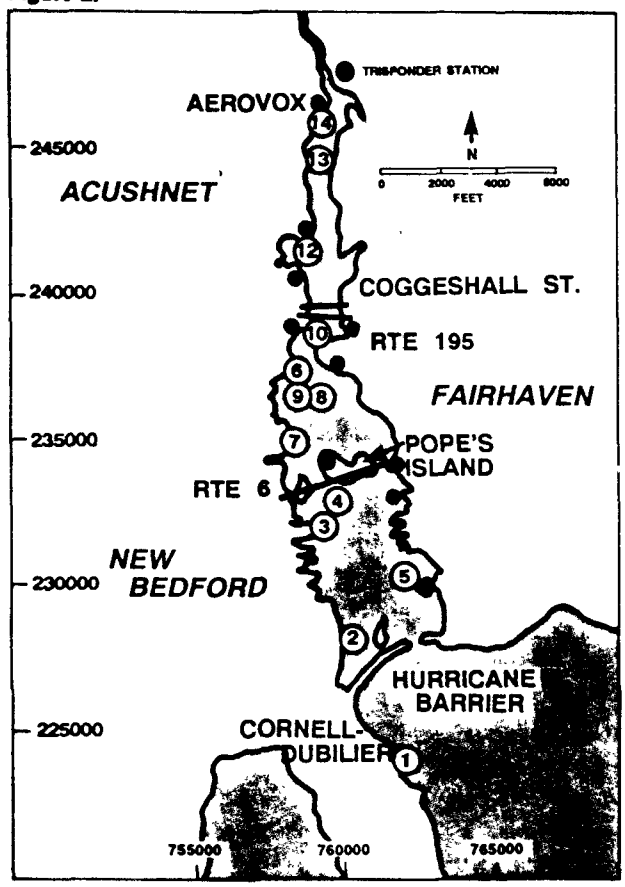
An example of how sediment data are used to define spatial gradients is drawn in Figure 1. These are test results for 10-day exposures to New Bedford Harbor, MA sediments using the amphipod *Ampelisca abdita*.

Figure 1.



These stations represent a gradient of PCB and heavy metal contamination increasing up the harbor from the lower-numbered to the higher-numbered stations (Figure 2). The mortality pattern clearly mimics the degree of contamination.

Figure 2.



Sediment toxicology is a relatively new field. Prior to 1975, sediment toxicity was generally inferred from field studies. In 1977, the EPA/COE developed an implementation manual that provided general guidelines for evaluating proposed dredged materials. Because the limitations of existing methods were recognized at that time, this manual was the impetus for sediment toxicity research and the development of standard methods within EPA as well as the COE's Dredged Material Research Program (DMRP). The early 1980s saw broadscale applications of sediment tests, particularly Swartz and colleagues' development of the *Rhepoxynius abronius* amphipod test method (Swartz et al., 1985). Further research has led to an

Figure 3.

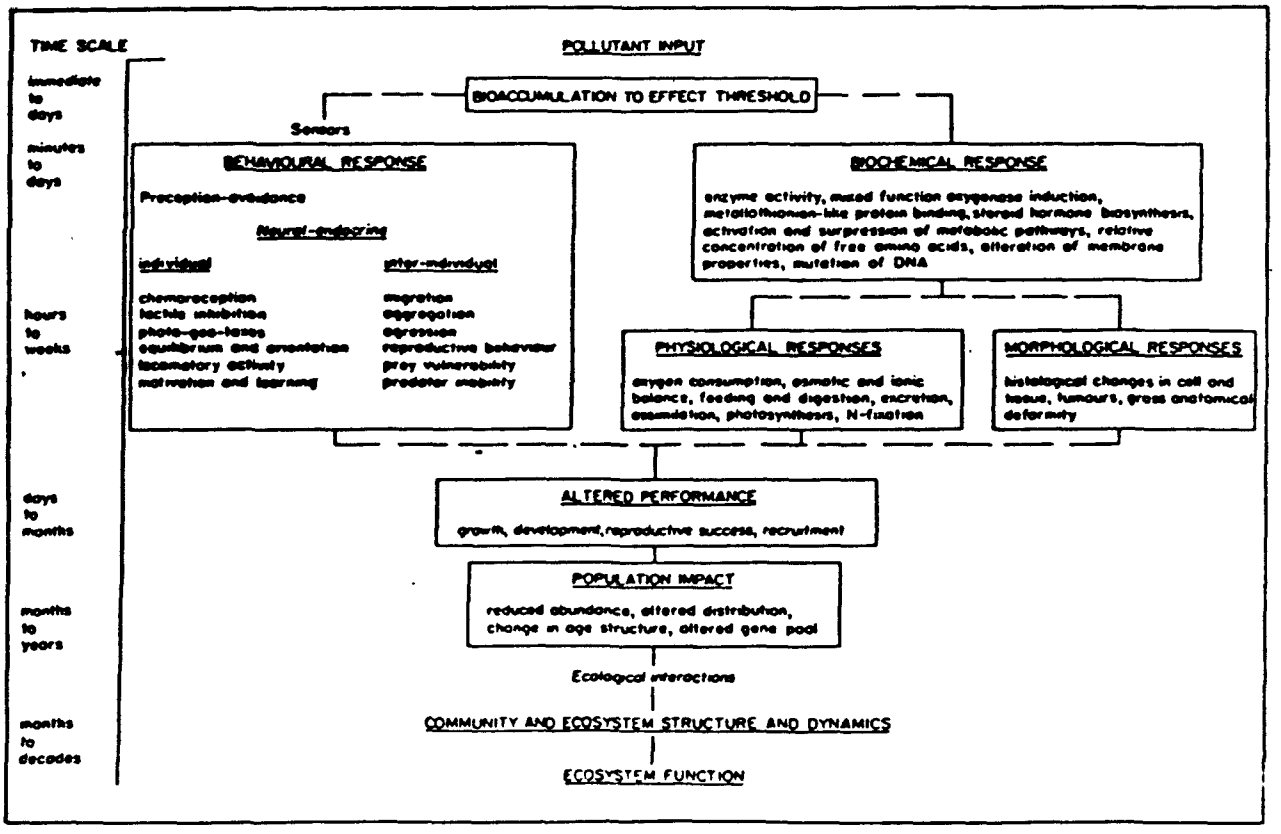
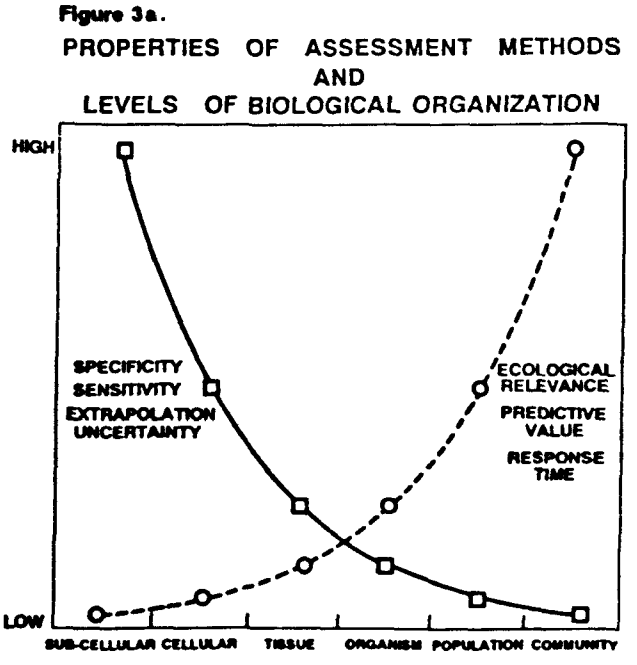


Figure 3a.



expansion of test species and endpoints, and the examination of modes of toxicity and contaminant availability. In the late 1980s we see widespread application of new methods, a growing understanding of modes of toxicity and the factors influencing availability in complex mixtures (i.e., synergistic and antagonistic effects), and the development of sediment quality criteria.

Exposure assessment and test applications

Several different media have been used in sediment toxicity tests. These include whole sediments, suspended sediments, pore waters, elutriates, and extracts. Methods of obtaining pore waters include centrifuging, squeezing, and applying pressure. Extracts may be prepared chemically using organic solvents. In most cases, whole sediment exposures

are the preferred method because pore water, extract, and elutriate methods suffer from interpretation problems. Suspended sediments are typically not an exposure route of concern.

Hazard assessment

Distinctions commonly used (often interchangeably) in hazard assessments are: acute vs. chronic (with the implication of time and an effects component); short- vs. long-term; lethal vs. sublethal; and screening vs. interpretative assays (with the latter implying the occurrence of a response to be interpreted). I suggest that the workgroup consider these definitions, but in any case, see Chapman (1989).

The range of biological responses that have been measured in tests with sediments spans the biological hierarchy. They include biochemical, cytogenetic, pathological, immunological,and physiological responses, as well as survival, growth, behavior, reproduction, development, metamorphosis, population growth, recruitment, and community structure. A series of screening level tests have been developed with bacteria (of which Microtox is an example) that examine genetic damage and bioluminescence. The types of biological responses that could be evaluated are summarized in Figure 3 (after Sheehan 1984). As one progresses up the biological hierarchy, the time scale of response increases from hours to years. In this progression, however, predictive value and ecological relevance also increase. Ideally tests should have reasonably short time periods but also have predictive value.

The EPA/COE Field Verification Program allowed for examination of a series of endpoints along this hierarchy using several different test species. In the most sensitive species tested, *Ampelisca abdita*, effects were seen on pathology, growth, survival, behavior, reproduction, and intrinsic rate of population growth. The latter responses were the most sensitive but also took approximately two months to develop.

Criteria for test organism selection

Test organisms should be selected on the basis of the following criteria:

- Ready availability either by culture or collection
- Wide geographic distribution
- Taxonomic relationship to site inhabitants
- Demonstrated sensitivity
- Ease of laboratory maintenance
- Ease of test method
- Ecological and/or economic relevance (most important)
- Compatibility with exposure/response matrix
- Insensitivity to geophysical factors
- Cost effectiveness

Available methods that may be appropriate to the Chesapeake Bay include the 10-day amphipod test, which has been used primarily in West Coast applications. One could also try several ranges of responses and tests, such as those proposed by Long and Chapman in the sediment quality triad using a suite of tests where various exposures and endpoints are evaluated.

Application to risk characterization

In order to apply sediment toxicity tests to risk characterization, we must determine the extrapolation potential of such tests. Specifically, (1) how do we extrapolate from a laboratory test to a field situation and (2) how do we extrapolate across the biological hierarchy from lower to higher levels of organization?

Both questions pose serious challenges. As far as data needs, extrapolation from the laboratory to the field requires good contaminant exposure information, which is based on local hydrography and circulation, and contaminant distribution. The biological resources at risk need to be identified; measured laboratory effects must be linked to these resources.

One quick example of the application of laboratory responses to field predictions using *A. abdita* is field verification at the Black Rock Harbor disposal site in Central Long Island Sound.

Figure 4.

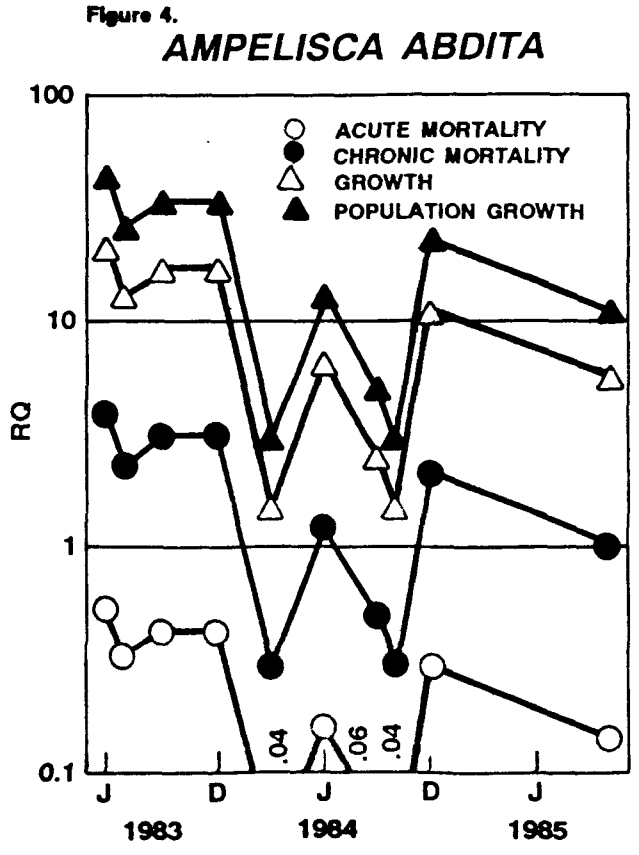


Figure 4 shows the ratio of predicted field exposures of contaminated suspended sediments to the effect concentrations for various responses. Exposure is defined and hazard levels are identified; they are combined using the quotient method (exposure/response) to derive risk predictions. This figure shows the potential for risk to each of the endpoints of concern. While these estimates have a great deal of associated uncertainty, the point here is that the approach and methods are available. To summarize, the toxicity test has to consider the type of organism, the response, and the exposure. The toxicity tests must link to field exposures and ecological resources in a predictive way.

Issues and research

Research must deal with sediment holding times, sediment manipulation, assumptions about chemical equilibrium, low-salinity tests, site specificity, and chronic test methods. We should also continue to investigate how to interpret and extrapolate suborganismal responses.

Questions

Q: Do we agree on cross-species sensitivity?

A: Two amphipods (*R. abronius* and *A. abdita*) have similar sensitivity; not many species have been tested for relative sensitivity.

Q: What about Microtox uses?

A: The problem is, what are you testing? If you're not using whole sediments, the interpretive perspective is lost.

Q: It seems that population growth is a more sensitive response than others.

A: This is true, but, as noted, these responses require a longer time frame. The combination of a series of responses together gives more complete information than any single response.

Q: How long until we have sediment quality criteria?

A: That's a good question, and the next speaker will address it.

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Contaminated Sediments and Sediment Criteria

Christopher S. Zarba

Introduction

Toxic contaminants in bottom sediments of the United States' lakes, rivers, and coastal waters create the potential for continued environmental degradation even where water column pollutant levels comply with established water quality criteria. The absence of defensible numerical sediment quality criteria makes it difficult to accurately assess the extent of the contaminated sediment problem. However, existing data demonstrate that there are many locations where existing and projected sediment contaminant concentrations are causing significant adverse effects to aquatic life and human health. The application of sediment criteria will make it possible for the States and the Environmental Protection Agency (EPA) to more effectively implement regulatory, enforcement, and cleanup actions where necessary.

Authority

Under the Clean Water Act (CWA) EPA is responsible for protecting the chemical, physical, and biological integrity of our nation's waters. In keeping with this responsibility, EPA published ambient water quality criteria for the 65 priority pollutants and pollutant categories listed as toxic in the CWA. While ambient water quality criteria are playing an important role in assuring a healthy aquatic environment, they alone have not been sufficient to ensure appropriate levels of environmental protection.

EPA has authority to pursue the development of sediment criteria in streams, lakes and other waters of the United States under sections 104 and 304(a)(1) and (2) of the CWA as follows:

(1) Section 104 authorizes the Administrator to establish national programs for the prevention, reduction and elimination of pollution by conducting and promoting "the coordination and acceleration of research, investigation, experiments, training, demonstrations, surveys, and studies relating to the causes, effects, and extent, prevention, reduction, and elimination of pollution and by publishing relevant information. Section 104(n)(1) specifically provides for the study of the effects of pollution, including sedimentation, in estuaries on aquatic life."

(2) Section 304(a)(1) directs the Administrator to develop and publish criteria for water quality accurately reflecting the latest scientific knowledge "on the kind and extent of all identifiable effects on health and welfare including, but not limited to, plankton, fish, shellfish, wildlife, plant life, shorelines, beaches, aesthetics, and recreation which may be expected from the presence of pollutants in any body of water, including groundwater . . . on the concentration and dispersal of pollutants, or their by-products . . . on the effects of pollutants on biological community diversity, productivity and stability, including information on the factors affecting . . . rates of organic and inorganic sedimentation for varying types of receiving waters."

(3) Section 304(a)(2) directs the Administrator to develop and publish information on, among other things, "the factors necessary for the protection and propagation of shellfish, fish, and wildlife for classes and categories of receiving waters. . ."

(4) To the extent that sediment criteria could be developed which addressed the concerns of the section 404(b)(1) Guidelines (for discharges of dredged or fill material under the Clean Water Act) or ocean dumping criteria (under the Marine Protection, Research, and Sanctuaries Act), they may also be incorporated into those regulations."

Approach to Criteria Development

A workshop was convened in which a wide variety of experts were given the responsibility of identifying a preferred method for generating sediment criteria. When potential approaches were being considered for their utility in providing an effective regulatory tool, it was understood that each approach had strengths and weaknesses and that any one approach would not be best for all situations. The participants of this workshop were provided with documents that:

- identified EPA's legal authority to develop sediment criteria and to regulate through their use, and
- identified a variety of methods that could be used in the development of sediment criteria, and
- identified the findings of a study that looked at contaminated sediments on a national basis.

A preferred approach was selected that many believed would provide EPA with the most effective regulatory tool. The Equilibrium Partitioning Approach (EP) was selected as the approach to be pursued to meet this goal. Over the past several years activities have been focused on evaluating and developing the EP for generating sediment criteria for regulatory purposes.

Description of Method

The EP focuses on predicting the chemical interaction between sediments and contaminants. An understanding of the principal factors that influence the sediment/contaminant interactions allows predictions to be made (based on sediment chemistry analysis) about the concentration of contaminant to which benthic and other organisms may be exposed. Data have demonstrated that the concentration of contaminant in the interstitial water (water between the particles of sediment) correlates very closely with toxicity and that the concentration of contaminant on the sediment does not. Chronic water quality criteria or possibly other toxicological endpoints, when compared with the concentration of contaminant in the interstitial water, could then be a reliable predictor of potential biological effects. The EP for generating sediment criteria focuses on predicting the concentration of contaminant in the interstitial water and compares that concentration to quality criteria. If the predicted sediment interstitial water concentration for a given contaminant exceeds the chronic water quality criteria for that contaminant then the sediment would be expected to be causing adverse effects.

The principal factors that influence sediment/contaminant interactions vary with the types of contaminants involved. Non-ionic, ionic, and metal contaminants interact with sediments in different ways. For non-ionic organic contaminants, predictions of sediment/-contaminant interactions are dependent on organic carbon as the principal factor that influences sediment contaminant binding. Sulfur compounds and organic carbon are some of the key factors that are being investigated that may influence binding between metal contaminants and sediments. Polar organic contaminants are currently being investigated to determine their chemical interaction with the sediments.

Specific Applications

Specific applications of sediment criteria are under development. The primary use of EP-based sediment criteria will be to assess risks associated with contaminants. The various offices and programs concerned with contaminated sediment have different regulatory mandates and thus have different needs and areas for potential application of sediment criteria. Because each regulatory need is different, EP-based sediment quality criteria which are designed specifically to meet the needs of one office or program may have to

be implemented in different ways to meet the needs of another office or program.

A likely mode of application of EP-based numerical sediment quality criteria would be a tiered approach. With such an application, the sediments would be considered to cause unacceptable impacts when contaminants in sediments exceed the sediment quality criteria. Further testing may or may not be required depending on site-specific conditions. Contaminants in a sediment at concentrations less than the sediment criteria would not be of concern. However, in some cases the sediment could not be considered safe because they may contain other contaminants above safe levels for which no sediment criteria exist. In addition, the synergistic, antagonistic, or additive effects of several contaminants in the sediments may be of concern. Additional testing in other tiers of the evaluation approach, such as bioassays, could be required to determine if the sediment is safe. It is likely that such testing would incorporate site-specific considerations. At the present time standard bioassays for assessing sediment contamination on a national basis are under development.

Current Use

The specific regulatory uses of EP-based sediment quality criteria have not been established. A review of the method for generating sediment criteria for non-ionic contaminants by the Science Advisory Board is ongoing. It is intended that this review be completed prior to the establishment of any formal framework for the application of sediment criteria. (The review of the method was held on February 2, 1989. The findings of this review are expected in September 1989.) The range of potential applications is quite large since the need for the evaluation of potentially contaminated sediments arises in many contexts.

Interim sediment criteria values were developed using the EP approach for a variety of organic compounds and were used to assist in the decision-making process in a pilot study involving six sites. These sites were Superfund sites that were involved with site characterization and evaluation activities. The interim criteria were used to:

- identify the extent of contamination
- assess the risks associated with the sediment contamination
- identify the environmental benefit associated with a variety of remedial options.

Potential Use

The EP method is likely to be useful in many of the activities being pursued by EPA. EP-based sediment quality criteria could play a significant role in the identification, monitoring, and cleanup of contaminated sediment sites on a national basis and in ensuring that uncontaminated sites will remain clean. In some cases sediment criteria alone would be sufficient to identify and to establish cleanup levels for contaminated sediments. In other cases the sediment criteria would be supplemented with biological sampling and testing or other types of analysis before a decision could be made. Sediment criteria can provide a basis for determining whether contaminants are accumulating in sediments to the extent that an unacceptable contaminant level is being approached or has been exceeded. By monitoring contaminants in the vicinity of a discharge, contaminant levels can be compared to sediment criteria to assess the likelihood of impact.

EP-based sediment criteria will be particularly valuable for monitoring sites where sediment contaminant concentrations are gradually approaching a criterion over time. Comparison of field measurement to sediment criteria will be a reliable method for providing early warning of a potential problem. Such an early warning would provide an opportunity to take corrective action before adverse impacts occur.

In many ways sediment criteria developed using EP are similar to existing water quality criteria. However, in their application they are likely to vary significantly. Contaminants at levels of concern in the water column need only be controlled at the source to eliminate unacceptable adverse impacts in most cases. Contaminated sediments have often been in place for quite some time and controlling the source of that pollution (if the source still exists) will not be sufficient to alleviate the problem. The problem is compounded by the difficulty and expense involved in safe removal, treatment, or disposal of contaminated sediments. For this reason sediment criteria are not anticipated to be used as mandatory cleanup levels, but as means for predicting or identifying the degree and spatial extent of contaminated areas so that regulatory decisions can be made.

Contaminated Sediment Committees

The development of sediment criteria using EP is only one of many Agency activities that address contaminated sediment problems. To ensure consistency and effectiveness in the development and implementation of these methods and procedures two committees have been established. These are the Contaminated Sediment Steering and Technical Committees. The principal roles and responsibilities of these committees are as follows:

Contaminated Sediment Steering Committee

develop long-term management strategy for contaminated sediments

facilitate the commitment of resources

establish policy/interim guidelines for managers

develop long-term research programs

explain/resolve inconsistencies in present sediment program activities

coordinate with other federal agencies

determine the role of economics in contaminated sediment strategy

identify changes needed in current statutes

Contaminated Sediment Technical Committee

coordinate technical activities

prepare guidance documents

prepare options documents

perform policy analysis

provide agency-wide technical support.

Questions and Answers

Q: What are the characteristics that affect release of contaminants?

A: They vary with different contaminants and different sediments. Many factors affect release; several factors dominate the amount of release. For non-ionic organic contaminants, organic carbon is the main factor. For metal contaminants, sulfur compounds appear to have the most influence.

Q: How do you define uncertainty?

A: We are in the process of doing that at this time. We will develop recommendations on this point by the end of this fiscal year.

Q: Can different types of carbon affect the availability of some contaminants?

A: Studies that we have done indicate that for the most part carbon is carbon with the exception of large particles like coal or other material. We are conducting additional investigations in this area.

Q: Have you planned to include a standard sediment test?

A: No, not for the purposes of developing criteria. There may be potential for using this technique when assessing variability in sediment methods in the future.

Q: Are biological tests run along with the sediment chemical analysis?

A: Yes, this is routine.

Plenary Session C:

Methodologies for Whole Organism Toxicity Testing

Convener: Lenwood Hall

Laboratory Testing of Ambient Receiving Waters

Steven C. Schimmel

Receiving water collection and holding techniques

Evaluating toxicity of ambient receiving water in the laboratory requires different techniques for collecting water in different settings. The most common technique is grab sampling. Another is composite collection, which uses a mechanical compositor to collect a sample over time. With both methods, the collector must assure the representativeness of the sample. Additional questions concern the different methods. Should I collect a grab sample at the surface or take multiple-depth collections? Will a collection of composite samples lose toxicity under the confined "artificial" conditions?

From our experience, ambient toxicity has been generally near-field in nature, and is generally not as widespread as we may expect. This is a result, in part, of toxicity decaying over time. If an effluent is the cause of toxicity, and it decays rapidly, then that toxic effluent component in the water will also decay rapidly. We therefore need to convey the sample from the collection point to the testing point as quickly as possible.

Sampling capabilities and options vary depending on the environment -- freshwater vs. saltwater, estuaries vs. streams. Small riverine situations are amenable to composite collection. They have accessible river banks for placing a compositor, and provide precisely identifiable locations. Flow conditions can also be helpful, as the collector may take advantage of unidirectional flow in deriving estimated effluent concentrations. Sampling on estuaries, large lakes, and large rivers is more difficult. Few stationary platforms are readily available, and for estuaries and lakes, hydrologic patterns are often uncertain or unknown. Exposure concentrations of chemicals or effluents in these systems are often vaguely defined, requiring dye studies.

These problems help us identify research needs for collecting ambient composite samples. We need compositors that maintain sample integrity, and the devices must be practical. They should be deployable *in situ*, function reliably in rough open water, and should be relatively inexpensive.

Sample collection, shipment, and holding conditions

Large volume samples may be necessary for toxicity testing.

Regardless of future research needs, all ambient samples should be held on ice for shipment to the testing facility. They should be placed in inert, unbreakable containers and kept in the dark. Tests should be conducted within 36 hours of collection. Remember that a receiving water may or may not be toxic, but whatever toxicity is there, you want to be able to detect.

Criteria for candidate test methods

One purpose of this workshop is to define a series of toxicity test methods for ambient waters of the Chesapeake Bay watershed. There are several criteria that should be included in that selection:

Sensitivity: The endpoint must be sensitive to low-level toxicity. It should include the most sensitive life-stages, and sublethal endpoints. The widely-used 96-hr acute lethality tests will not be sensitive enough.

Efficiency: Tests should be quick, require a low volume of water, and be cost-effective.

Relevance: The species chosen should be relevant to Chesapeake Bay, if at all possible. Ideally, a suite of phylogenetic groups could be selected that represent the biota in the Bay. Species that occupy important ecological niches would be particularly desirable.

Recommended species

Freshwater:

Green alga - *Selenastrum capricornutum*

Duckweed - *Lemna minor* (number of fronds and chlorophyll content)

Daphnid - *Ceriodaphnia dubia* (growth, survival, and reproduction including "r " and time to onset of reproduction)

Fathead minnow - *Pimephales promelas* (growth, survival)

Saltwater:

Diatom - *Skeletonema costatum*

Red macroalga - *Champia parvula* (sexual reproduction)

Sea urchin - *Arbacia punctulata* (2-hr test, fertilization)

Bivalve larvae test (48-hr test) - *Crassostrea, Mytilus* and *Mercenaria*

Mysid - *Mysidopsis bahia* (growth, survival, fecundity) - 7-day labor-intensive test

Inland silverside - *Menidia beryllina* - the only species that can tolerate the salinity range of the entire Chesapeake Bay (growth and survival)

Sheepshead minnow - *Cyprinodon variegatus* (growth and survival)

Precision testing has been conducted for most of these species to test reproducibility. Where there are data, variability was relatively small (30-50%). These methods have been used to test ambient waters and effluents in mobile laboratories around the country.

Regulatory history

In addition, all the methods listed above have been used for regulatory purposes, that is, either in the NPDES permitting system, for effluent toxicity limitations, or in pesticide registration. They will likely be used in some Superfund applications.

Acute lethality tests have the longest track record. Most of the acute lethality methods have been subjected to inter- and intra-laboratory testing programs for precision testing. Most methods have received legal challenges, and these challenges are ongoing.

Ambient *in situ* salt water methodologies

Evaluating the toxicity of ambient waters directly *in situ* provides obvious advantages over laboratory evaluations. The blue mussel, *Mytilus edulis*, has been used for that purpose for over ten years. The blue mussel has a range that borders on the Chesapeake Bay. About 100 organisms are placed in each basket and suspended in the water column, where they are kept *in situ* for at least one month. Measurements of growth and survival are taken. In addition, laboratory toxicity tests such as "scope for growth" can be done immediately following field exposure. Scope-for-growth measurements obtain an energy budget for the animal to determine if energy is available for growth and reproduction, over and above that needed for basic metabolism.

In an *in situ* study, you have to make sure that you match physical conditions such as salinity, temperature, and food availability in order to make inferences about toxicity among stations.

Questions

Q: Does the insensitivity of *Cyprinodon* extend across all chemical classes?

A: No. You can't make any hard and fast assumptions on sensitivity, which is very specific to the experiment and the chemical that the species is being exposed to. Relatively speaking, *Cyprinodon* and *Menidia* are at the low end of the sensitivity scale. For this reason and because of the high labor intensity of this experiment, this test may not be worth the effort, depending on specific program needs.

Q: Would you object to the use of *Neomysis americana* as opposed to *Mysidopsis bahia*, since *Neomysis* is more relevant to the Bay?

A: No, I don't have any objections. However, we were not as successful at culturing that species as we were with *Mysidopsis*. There is still much basic work to be done to better understand optimum culture techniques. The listing I provided should simply lay the groundwork, and I would expect that a list of recommendations for research initiatives on additional species will come out of this workshop.

Q: Is there a database on the relative sensitivity of the organisms that you presented?

A: Yes, there is one for the saltwater species I discussed, with the exception of the bivalve larvae.

Q: Have you compared the reproductive indicator, the 7-day *Mysidopsis* test, to the results you get in the 28-day test?

A: Those comparisons have been conducted for at least 10 different organic and inorganic chemicals independently; a large majority show close correspondence (i.e., good predictions of life cycle effects).

Q: Have you ever seen effluents or receiving waters increase in toxicity over time?

A: No, I have never seen it.

Q: Are you suggesting a battery of tests as opposed to a tiered approach? And what are the advantages and disadvantages of each?

A: We are evaluating toxicities in ambient receiving waters, which do not integrate toxicity. They may appear to be spurious or concurrent with episodic events such as storms and the like. Resuspension of sediment material may cause some toxicity. You're talking about short-term exposures and endpoints that relate to life cycles. Also, using a suite of organisms that occur naturally is the best way to go.

A tiered approach, as is used for pesticide registration, is good for a single, conservative chemical. I don't think it is good for ambient water that is not conservative. The group can decide whether that is the best approach or not.

Q: How many chemicals would we need to test to get a good idea of the effects?

A: I would rather look to the classes of chemicals, i.e. organic chemicals with

high vs. low octanol:water partition coefficients, metals, surfactants. Also, I'd use those chemicals shown to cause environmental problems.

Q: Wouldn't it also be useful to use a benchmark toxicity test -- for example, tests that we have a large database on, to run side by side with the selected methodologies for a few times?

A: Yes, one that's been used in the regulatory process successfully.

This group should be aware of EMAP -- Environmental Monitoring and Assessment Program. This monitoring program will be carried out next summer in a pilot study at a series of stations from Cape Cod to Cape Hatteras including several stations in the Chesapeake Bay. I would propose that there be good communication between this workshop and the EPA/NOAA EMAP program. It only makes sense to do so.

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Field Toxicity Testing Procedures

Jeffrey Black

I will be discussing some background and techniques concerning field testing used both on site and *in situ*. In the amount of time allotted, I will not be able to cover all the methodologies used. However, I have provided a rather extensive bibliography if more detail is required. I've put some special emphasis on the embryo-larval survival and teratogenicity test, because Steve Schimmel asked me to describe that procedure as it is used in both the laboratory and the field.

For purposes of discussion, the definition of "on site" is testing performed in the field using mobile facilities -- in other words, bringing the lab to the field site. *In situ* tests are those conducted instream.

Toxicity testing vs. chemical monitoring

There are advantages to performing ambient toxicity testing as opposed to collecting only chemical monitoring data. My preference is to combine both chemical monitoring and biomonitoring when possible, so that if toxicity is demonstrated the cause may be discernible. But cause-effect relationships are not always easily established. To choose between the two kinds of monitoring, certain aspects of each must be considered.

First, we must consider that water quality criteria have been established for only a few chemicals present in the environment. Second, the chemical approach alone likely will not detect all the chemicals in a contaminated system. Although chemical monitoring is appropriate when only a few chemicals are known to be present in a system, it does not suffice when many chemicals are present, especially considering possible synergistic or additive toxicant interactions. In these situations we use organisms to "see" those chemicals and chemical interactions in the effluent or in the receiving water.

We can make assessments of bioavailability, temporal variability, and persistence of toxicity by using biomonitoring. For determining the presence of bioaccumulative chemicals, both chemical monitoring and toxicological monitoring are appropriate, especially if the term "toxicological" can be extended to encompass bioaccumulation studies. For rapid assessments, chemical monitoring is probably more efficient for detecting carcinogens, although some pathologists might disagree, arguing that we can look at indigenous aquatic organisms for tumor formation and other biomarkers. Both chemical and toxicity assessments are reliable for design of treatment systems (e.g., chlorine reduction). In selecting methods, of course expense must be considered; you have to weigh what you want to do against the availability of funds.

Purposes of testing

The purposes of ambient testing vary; different tests are used to accomplish different objectives.

Some can be used for regulatory purposes. Many times an industry's discharge permit is based on characteristics of the mixing zone of the stream. Ambient toxicity testing could be used to determine compliance in the mixing zone.

We may want to develop a scientific data base so the findings of toxicity tests can be correlated with ecological data to validate biomonitoring endpoints.

Ambient tests may be used to determine sources of impact. For industries with only one or two discharges, the sources are obvious. For nonpoint sources such as agricultural runoff, ambient testing can be useful in tracking important toxic inputs.

We can also assess temporal variability and the persistence of toxicity from a source (spatial variability).

We can evaluate the effects of water quality characteristics (e.g., salinity, hardness, pH) on toxicity.

Selection of field stations

The selection of field stations includes a number of important considerations. First of all, choosing a station using map siting alone is usually unsatisfactory, as it does not indicate many limitations of access. The proximity of stations to impact sources is also very important in attempting to determine if there is any spatial variability in effect. The establishment of a control or reference site can be difficult. For instance, an upstream site may be contaminated to the extent that it is unsuitable. Thus we may be restricted to using lab control water (reconstituted water). The sites should be evaluated as to their importance as spawning areas or recreational areas, and for their potential for producing human health effects. For field validation studies using benthic invertebrates, fish populations, periphyton, etc., sites for ambient testing should be selected close to the ecological sampling sites.

The types of tests to be used -- acute, chronic, or "short-term" chronic -- must be determined. In many cases dealing with ambient toxicity, acute tests are not adequately sensitive. Whether instream chambers or mobile labs are used, the sampling procedures, selection of test organisms, cost, and personnel needs are all important, and these considerations have been addressed by earlier speakers.

Variations in toxicity between lab and on-site evaluations of effluents often have been observed. Generally, lab tests on stored effluents are less sensitive, in some instances by up to two orders of magnitude (Birge et al. 1985). In such cases, field testing with a mobile facility has advantages over lab tests.

Standardized on-site test procedures vary. Acute tests have been well documented in *Methods for Measuring*

the Acute Toxicity of Effluents to Freshwater and Marine Organisms (Peltier and Weber, 1985). Chronic short-term tests for ambient waters thus far validated to some extent are the fish embryo-larval survival and teratogenicity test, fathead minnow larval survival and growth test, and *Ceriodaphnia* survival and reproduction test (Birge et al., 1989; Mount et al., 1985, 1986).

Endpoints

As new tests are developed, reliable and reproducible test endpoints must be established. The comparability of values from short-term tests to results of longer-term tests must be addressed. We compared LC_1 (threshold) values with fish species to MATC values for similar species in longer chronic tests and found them to correspond in many cases (Birge et al., 1985). In embryo-larval tests, teratogenicity is incorporated with mortalities in calculating NOEC, LOEC, and LC values, and these tests are applicable to both freshwater and saltwater species.

Several studies show that data from ambient toxicity tests correlate to ecological stream parameters. More work is needed in this area. If we don't know what the toxicological endpoints mean in terms of the environmental impacts, what good are they? So we have compared embryo-larval test results with other ecological parameters. We have found highly toxic situations in which receiving water killed all the test organisms. We noted graded responses downstream to a point where the survival range in the toxicity tests was comparable to that observed for the control area water. Studies revealed that the number of macroinvertebrate taxa also correlated well with embryo-larval survival frequencies.

Similar tests in mobile labs have incorporated several marine species (Schimmel et al., 1989). Reproduction of the red algae was the most sensitive endpoint, and the survival and growth of sheepshead minnow larvae were the least sensitive. Observed effects were attributed to ammonia from a pulp and paper mill.

Instream evaluations include:

Juvenile fathead minnows (~250-350) in chambers were used to study bioaccumulation of PCBs in the Hudson River (Jones and Sloane, 1989).

In the upper Chesapeake and the Choptank River, caged striped bass larvae and yearlings were deployed from rafts, and composite chemical samples were analyzed in conjunction with observations of bass mortality (Hall et al., 1988).

Using shorter-term tests, Borthwick et al. (1985) and Clark et al. (1987) studied pink shrimp after the field application of a mosquitocide (fenthion). Mortality was observed within a 24-hour period, and field observations confirmed effects seen in laboratory studies.

A longer-term test was performed by Freeman and Sangalang (1985) using the Atlantic salmon over a 3-month period, with sampling conducted every 2-3 weeks for mortality and weight determinations. The fish were collected at sexual maturation and the differences between fish in acidic vs. non-acidic conditions were determined. Fish taken from acidic areas gained less weight, produced smaller eggs, and showed abnormal metabolism of steroid hormones.

Community toxicity tests

Community Toxicity Testing (ed. Cairns, 1986) is a good reference (e.g., see Lewis et al., 1986).

Clements et al. (1988a) used artificial substrates for studying aquatic insect communities. Substrates were placed instream where ambient toxicity was expected. Diversity and density were measured after 30 days.

Lewis et al. (1986) looked at the phytoplankton and periphyton in stream and lake systems, adding a toxicant back to the natural system. This procedure is somewhat different from the type of testing proposed for the Bay, but certain adaptations to this method may be useful.

We used periphyton samplers (microscope slides placed on a floating tray) in ambient water and compared impacted areas for differences in density and diversity. Sometimes we had major problems when flooding removed many of our samplers, and data collection was severely hampered. However, these techniques can be very efficient in ascertaining ambient water quality.

Problems

Other problems relate to situations when the water taken from reference or control areas is toxic. Also, we can change the integrity of samples when collecting for on-site testing. For example, using standardized procedures, we are generally limited to controlling temperature. Thus if temperature is a problem in the ambient system, the standardized tests can't provide this information. In this case, in situ procedures may be more appropriate.

We need to return to examining the effects of natural perturbations. Conventional pollutants must be assessed, including suspended solids, chlorine, pH, and oil and grease. With multiple discharges, it is difficult to assess cause-effect relationships in ambient systems where toxicity is low and only occasionally observed. Furthermore, we must evaluate the ecological importance of toxicity endpoints. Statistical procedures for interpreting results exist in manuals for traditional types of toxicity testing, which use a graded concentration range and "typical" controls.

How valid are these types of standardized statistics in determining the significance of ambient toxicity? Should toxicity in ambient systems be compared to reconstituted control water? Does statistical significance equal ecological significance?

What happens when you conduct a battery of tests -- two showing toxicity problems, two showing no problems, and two that don't work? This type of situation can often occur, when data from test batteries are conflicting.

I have no argument with looking at more than one species, but the organisms must be carefully selected and their importance validated. Research should focus on more critical comparisons of test endpoints to actual ecological effects. We also need to reassess existing data and re-evaluate raw data from published validation studies. Perhaps incorporating a combination of standardized and caged-organism testing would be useful. I think we need not try to protect our own turf any more, but rather talk with each other and develop and integrate more approaches to ambient toxicity testing.

Questions

Q: Have you used a reference toxicant with a known effect (LC50)?

A: In response to the reference toxicant, yes, we routinely use cadmium to assess variability of the responses of organisms and the quality (viability) of the organisms. The reference test with a chemical of known toxicity is an important aspect of field work, especially when using standardized procedures.

Comment: A cautionary note concerning the caged test: it really depends on the individual species as to the variance of metal response and uptake. We did an acid-mine drainage-site study on caged trout. The trout looked fine on the superficial level, but when we looked at the subtle things like metal metabolism at

the cellular, subcellular, and tissue levels, the caged organisms, whether at control or impact sites, had very different responses. The responses varied particularly in uptake of metals, where the metals were deposited, and susceptibility to metal toxicity. Other species of fish were not very sensitive to it. We must be careful in interpreting *in situ* tests, because some species really don't like being caged.

Response: These considerations are important relative to assessing toxicant-related vs. testing-related responses.

Q: How far apart are your testing stations?

A: It depends. In one study, we were dealing with more than 40 miles of stream from the effluent source to the last ambient station. In other studies, the distance was much less. Station locations are specific to the study site.

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Plenary Session D:

Methodologies for Suborganismal (Biochemical and Cellular) Toxicity Testing

Conveners: Jay W. Gooch, Peter Van Veld

Gooch: I appreciate Dr. Black's suggestion that we need to break down some of the barriers in our thinking between ecological endpoints and subcellular endpoints. I think that those of us studying the subcellular level do have a contribution to make to the overall discussion.

For this afternoon's presentations, Dr. Roesijadi will begin with more of a philosophical discussion of biochemical endpoints; where they've come from, expectations about the information they will provide, and limitations to linking the endpoints to population or community level impacts. I will follow up with more detailed information about some of the potential measures and demonstrate the kind of information they can provide.

Rationale for and Relevance of Analysis of Suborganismal Responses to Contaminant Exposure

G. Roesijadi

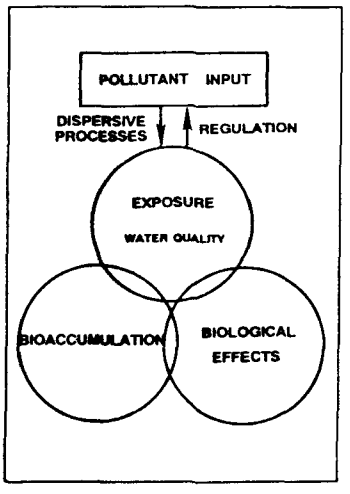
A major question regarding the analysis of suborganismal responses to contaminant exposure is "Why has an approach with the power and ability to solve problems in human health not been readily adopted to solve problems of environmental health?" If we understand suborganismal responses -- both the normal functions and deviations caused by pathological conditions -- we can use that information to deal with problems related to the exposure of organisms in the environment. Understanding the mechanisms of toxic action can provide tools for detecting and assessing a toxicant's effects. However, the general premise of such a clinical approach to assessing environmental health has not yet been accepted.

The recent history of this field goes back some 15-20 years when biologists began to actively pursue a greater understanding of the mechanisms underlying the effects of environmental contaminants in aquatic organisms. The concept of a diagnostic approach was adopted and has been variously referred to as sublethal effects, biochemical indicator responses, biological effects monitoring, and, currently, biomarkers. These synonyms reflect attempts to establish an identity for a developing field of environmental science.

General Framework for Biological Effects

At the present time, we know a good bit about measuring pollutant inputs and dispersive processes (Figure 1).

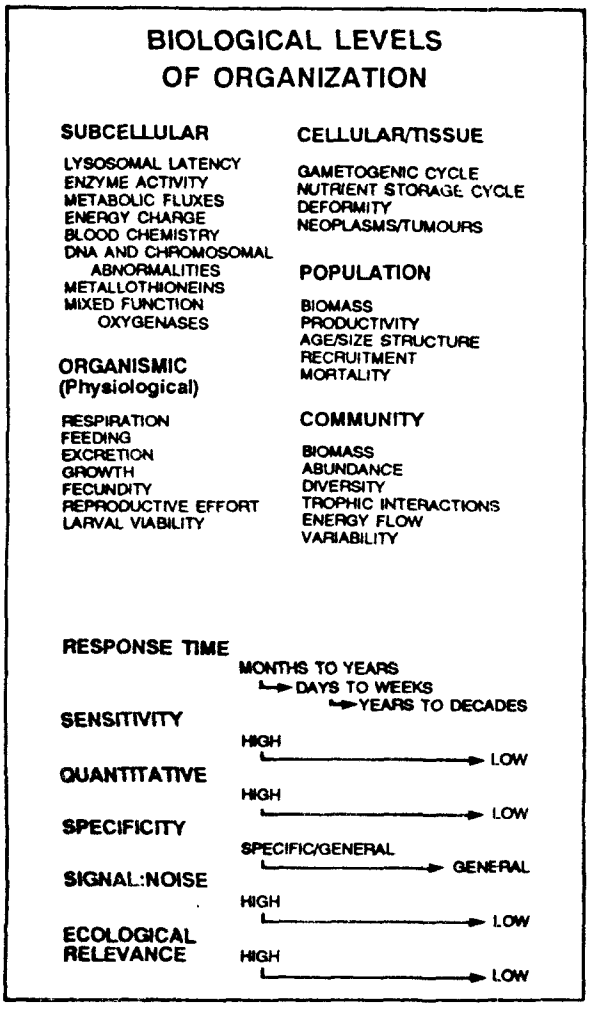
Figure 1.



In the realm of analysis of environmental processes, we are able to deal relatively well with describing the chemical environment and bioaccumulation by organisms. Conceptual frameworks and analytical procedures associated with measuring these processes are generally well-accepted. We do not have a good understanding of the means for assessing biological effects. If we did we would not have convened a forum such as this in which the merits of design of assessment and monitoring programs are being discussed. We are here because we need to assess approaches to take and need to come to a consensus on which available ones will be productive.

By suborganismal responses, we mean responses that are mainly at the cellular and subcellular levels of biological organization (Figure 2).

Figure 2.



If one looks at biological levels of organization from the subcellular to the community and examines parameters such as response time, sensitivity, ability to quantify, specificity, signal-to-noise ratio, and ecological relevance, the more detailed levels of organization can provide a higher degree of rapid, sensitive, and quantifiable information. However, the ecological significance of such measurements is considered low. At the more complex levels of organization where ecological relevance is high, the picture becomes blurred as signal-to-noise becomes less evident and the time-scales required to obtain information are long. One of the difficulties in the study of suborganismal responses is the problem of relating information derived at lower levels of biological organization to more complex levels; in other words, the ability to extrapolate effects from lower to higher levels of organization.

Ecological Relevance of Suborganismal Responses

In the past, we have tended to view all levels of biological organization in a nested fashion (Figure 3 top).

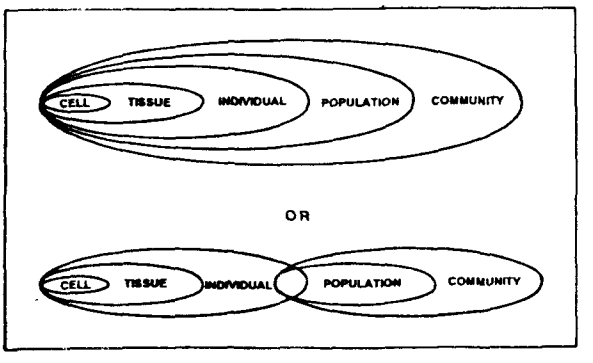


Figure 3. Perceived relationships of various categories of biological organization (top figure from Molecular Ecology Institute, California State University, Long Beach).

Strict adherence to this view presents a problem, because it implies that processes at molecular and cellular levels can be directly extrapolated to populations and communities. There is a demand that measurements of cellular processes be extrapolated to population- and community-level processes and thus

have ecological significance. I think that processes measured at the suborganismal levels are ultimately useful in explaining responses at the individual level. They can reflect on the population in so far as the measurements being made are representative of responses of individuals in a given population. Extrapolation of such measures to population processes is debatable. Thus, another representation of the relationship between the various levels of organization is presented in Figure 3 (bottom) in which the suborganismal and organismal processes are less directly coupled to the population and community, although a relationship is recognized. We have to ask whether processes such as reproductive output, which is considered to be an ecologically-relevant measure, are directly coupled to recruitment, which is a population parameter. The relationship may not be direct. Recruitment processes are not well enough understood for us to make unequivocal statements about the relationship between reproduction by individual organisms and population responses. Arguments that relate to density-dependent and -independent controls would also be applicable. If we understand processes at the more detailed levels of biological organization represented by suborganismal responses and have enough information on their relationship to organismal function, we can extrapolate to processes that ultimately relate to organismal functions such as growth and reproduction. Other factors, referred to as emergent properties by some, may then control how the individual relates to the population and community.

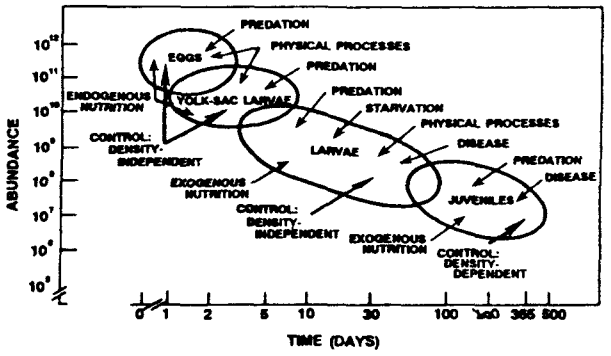


Figure 4. A conceptualization of factors that influence the abundance of fishes during the recruitment process (from E. Houde, 1987, Fish early life dynamics and recruitment variability, Am. Fish. Soc. Symp. 2:17-29).

A figure on recruitment (Figure 4) from a paper by Dr. Edward Houde shows that many factors can influence the actual numbers recruited into a fish population. He has identified the period of larval development as one that is most sensitive to changes in environmental parameters and other factors such as predation and food availability.

Figure 5.

HYPOTHETICAL RECRUITMENT OF YOUNG FISH UNDER ONE GOOD AND THREE BAD CONDITIONS (±25% CHANGE IN MORTALITY OR GROWTH RATES) (HOUDE, 1987)

CONDITION	INITIAL n	MORTALITY (d^{-1})	AGE AT METAMORPHOSIS (DAYS)	RECRUITED n
GOOD	$1x10^{6}$	0.100	45.0	11,109
BAD-1	$1x10^{6}$	0.125	45.0	3,607
BAD-2	$1x10^{6}$	0.100	56.2	3,625
BAD-3	$1x10^{6}$	0.125	56.2	889

FIGURE 5 TABLE ON HYPOTHETICAL RECRUITMENT OF LARVAL FISH UNDER VARIOUS CONDITIONS AFFECTING GROWTH AND SURVIVAL (FROM E. HOUDE, 1987, FISH EARLY LIFE DYNAMICS AND RECRUITMENT VIABILITY, AM. FISH SOC. SYM. 2:17-29).

In a table of hypothetical recruitment (Figure 5), he gives four examples that include one with "good" recruitment conditions and three with "bad." The three bad conditions were a 25% increase in mortality, 25% increase in age-at-metamorphosis (a reflection of growth rate), and both simultaneously. These "bad" conditions can occur independently of any pollution-related processes and cause significant reductions in numbers recruited. If the "Initial n" in the table is altered to simulate changes in reproductive output, using the same values for mortality and age-at-metamorphosis as in the good condition, very large changes in "Initial n" would have to occur to cause reductions in recruitment similar to the "bad" conditions. Thus, to obtain reductions in recruitment equivalent to "bad" conditions 1 and 2, a 68% reduction in "Initial n" is needed. To achieve reductions equivalent to "bad" condition 3, a 92% reduction in "Initial n" is needed. The effects of such reductions as a result of contaminant-related effects may not be detectable in the context of natural variability in recruitment. Coupling responses at the individual

level with population processes can be extremely difficult. Demonstrated effects on "ecologically-meaningful" measures such as growth (of inappropriate life stages) and reproduction in an experimental context may have little meaning in the real world.

If we can accept the premise that it is very difficult to couple lower and higher level processes directly as one goes from the individual to the population, then suborganismal responses will have a different frame of reference and take on new meaning. It must be recognized that the demand for extrapolation to population processes will most likely not be met when using suborganismal responses. Therefore, the value of responses of individuals would have to carry more weight in environmental assessment than they currently receive. Diagnostic and clinical approaches would then increase in relevance, having meaning at the individual level. Proper diagnosis and appropriate sampling procedures can provide measures of the presence and extent of adverse change within existing members of a population. This information in itself should be of value for those needing to make immediate decisions relating to environmental contamination and its ramifications. The limitation of uncertain ecological extrapolation should not deter us from using an approach with the greatest potential for early and sensitive detection of contaminant-related effects. As in the detection and diagnosis of sick humans by the medical profession, there should be intrinsic value in the detection and diagnosis of sick animals in the natural environment.

Summary

The knowledge that individuals are adversely affected by exposure has intrinsic value and should be used in environmental management. The expectation of extrapolation of measures based on individuals to ecologically-significant processes is currently unrealistic.

Diagnostic approaches known to be effective in epidemiology and medicine will be effective in environmental toxicology.

However, to effectively utilize such approaches, we will have to reevaluate the value system that assumes that effects must have demonstrated ecological significance for them to be valid measures of biological effects and suitable for management decisions.

Questions

Comment: It is difficult to go from organism to population, but it is important to understand that much of what we do is based on the individual organism.

Comment: I disagree. It is not true that populations are uncoupled from individuals. Populations of some species (r-selected) are driven by environmental conditions, but many (including some that society is particularly concerned about, like striped bass) are not. A 68% reduction of reproduction rates is not high.

Response: But this is about the same level of reduction that was produced by changes in mortality or longevity of larval stage; this was the point. Natural changes can be too small to detect but make very large changes in recruitment. The other point is that matters like coupling are highly controversial in the ecological field.

Comment: No more controversial than the prediction of individual responses from biomarkers.

Response: The point was that in dealing with organismal responses, the demand of extrapolating to population responses may be unrealistic. "Biomarkers" is for me a red flag. I think we can accept that cellular processes do affect fitness of individuals -- we accept it in humans, anyway. The connection may not be

well demonstrated in the species we study, but that doesn't mean it is nonexistent.

Comment: The linkage between individual and population responses can be variable, depending on environmental/ecological factors, which may be stronger in some years than in others. It has to be viewed in the context of the natural environment.In doing some preliminary scope-of-growth studies it occurred to me that the fecundity level shows the energy level built up over a few weeks, and some of these tissue level tests can be done over a couple of days. So we see some integrative function from subcellular tests, and some things that may be early warnings. The problem is quantifying what finally happens to the animals. The quantitative links must be established by future research.

Techniques for Assessing the Sublethal Effects of Chemical Contaminants on Aquatic Life

Jay W. Gooch

The use of biomarkers and their integration into routine testing schemes has so far met with resistance. The regulatory and management communities don't necessarily understand how this approach helps them. It is the responsibility of the research community to make the importance of this level of information apparent and to communicate realistic expectations about the information provided. This is an emerging technique, and the question is whether this extra information gives us anything we need to know.

One of the original justifications for the use of sublethal endpoints in environmental testing was the desire to have an early warning system. It was recognized that by the time resource managers could detect declines in populations, the damage to the resource had already occurred.

One of the problems we encounter in the use of biochemical endpoints in environmental research is the emphasis on population or community level responses. These measurements usually come into use through progress that is made in mammalian research where we place an intrinsic value on the individual response, largely because we are trying to protect the health of individual humans. By contrast, in environmental research we do not place our emphasis on the individual; hence, there is a resistance to studies focused at this level.

I have listed a number of the justifications that have been put forth for the use of biochemical endpoints. These endpoints:

- assess the "health" of animals, serving as diagnostic tools.
- provide early warning of habitat degradation.
- integrate multiple exposures and interactions.
- account for modifiers of toxicity.
- can serve as measures of exposure.
- can be useful in tracking environmental recovery.

One of the initial issues to discuss is specific vs. general measures. In the literature you will find that there is no general agreement about this issue, and there is not enough time here to discuss the arguments in great detail. Rather, I will discuss some of the advantages and disadvantages of each. Clearly, depending on the circumstances and the questions being asked, there may be merit to one approach or the other, or some combination. In general, I don't think anyone would suggest that there is any one magical biomarker that will provide information under all circumstances.

Specific vs. general measures

Specific tests provide a strong mechanistic interpretation, i.e., links between cause and effect. Responses can be grouped by compound class as related to mode of action. One of the problems, however, is that information is lacking for some groups of compounds, or the mode of action leaves unknown changes at sublethal levels (e.g. cyclodiene insecticides).

Another argument in favor of specific measures is that there is a large mammalian data base to draw from. As I stated earlier, many of the measures we use have evolved out of studies done in mammalian systems, where the mechanistic approach to toxicology prevails and consequently these measures are generally more developed.

Some examples of specific measures are induction of cytochrome P450-mediated monooxygenase enzymes by certain organic pollutants, induction of metallothioneins (metal-binding proteins) by metals, inhibition of acetylcholinesterase by organophosphate and carbamate insecticides, and inhibition of delta aminolevulinic acid dehydrase by lead. Clearly, this is not an all-inclusive list.

The greatest strength of general responses is that they are ideally sensitive to a wider group of stressors. However, we usually have less background information on "normal" ranges for these parameters and understand less about how they are affected by natural environmental variables. In addition, there is less mechanistic interpretation or cause/effect linkage possible. For a manager this means that the measure by itself will not be able to suggest any particular contaminant. More information would be needed in order to determine where regulation or enforcement should be enhanced. I am not suggesting that specific measures provide all of the information that is needed either. The point is that the information gained is directly related to the tools that are used.

Below I have listed a number of different general measures of response. My bias is to lump many of the measures that others consider more whole-organism/physiological measures into this category of general measures of response. I won't discuss any of these in great detail as time does not permit. Recognize that this is only a partial list. A good summary of many of the enzymes or measures that have been used can be found in Neff (1985).

Examples:

RNA/DNA ratios

adenylate energy charge

glycogen levels

scope for growth

amino acid profiles

serum chemistry (important in mammalian systems)

histopathology

others

Because the preliminary documents that were distributed contained a list of some of the candidate measures for this effort, I tended to focus on them in my preparation. Again, this is not intended to be an all-inclusive list.

Candidate measures

I would like to preface this part of my talk to reveal my bias up-front. The cytochrome P450 enzymes are one of my research areas. I am going to dwell on them somewhat, not to sell you on them as the only relevant parameter to measure, but rather to use them as an example of the kinds of data that are being

generated from field studies using subcellular measures. Also, I think they represent a useful example of the kinds of things that need to be considered when determining the significance of these kinds of measures.

Toxification and detoxification enzymes. In the general reaction scheme of the primary and secondary biotransformation pathways, the first reaction is often referred to as a phase I transformation. This is the initial reaction required to transform a lipophilic molecule to one that is more water soluble, or to alter the molecule so that it can be further metabolized to a water-soluble form by secondary or phase II reactions. The first reaction is catalyzed by the cytochrome P 450-mediated monooxygenase system. One view suggests that this family of enzymes has evolved in response to the need to protect the body against potentially harmful foreign chemicals, particularly those entering via the diet. The attribute of this system that provides a useful measure in an environmental context is that specific forms of P450 enzymes (detected as activity or protein) are induced by chemical exposure. In other words, when animals are treated with various chemicals, the transcriptional machinery of the cell, particularly the liver cell, is activated, and increased amounts of specific forms of P450 enzymes are synthesized. It is this enzyme induction, as it is referred to, that provides a specific measure of exposure.

Without going into too much detail, let me just tell you that fish respond in a much more limited way than do mammals. Kleinow et al. (1987) list a number of the fish species that have been investigated and the compounds that they have shown a response to. The important thing to note is that fish P450 enzymes respond consistently only to the planar, and often halogenated, chemical contaminants. Fortunately or unfortunately, depending on your point of view, these compounds are some of the more ubiquitious and toxic pollutants we have in aquatic systems. These pollutants are the PCBs, PBBs, PAHs, dioxins, dibenzofurans and the aromatic fraction of petroleum.

There are several reasons why the functional status of this system may be important to an animal. For a number of compounds, metabolism is an activation process, i.e., the metabolic product is more toxic than the parent compound. In addition, many metabolites, particularly those of PAHs, are carcinogenic. Hence, the status of P450 enzymes may play a determinant role in the effects of a chemical on an organism. For example, Dr. John Lech's laboratory (Erickson et al., 1986), studied rotenone toxicity under various conditions of the P450 system in rainbow trout. Under normal (i.e., control) conditions, rotenone exhibits a 96-hour LC50 of approximately 3.5 parts per billion. When the fish were treated with beta-naphthoflavone, an inducer of P450 activity, rotenone was actually less toxic, with an LC50 now near 4.5 parts per billion. In addition, when fish were treated with piperonyl butoxide, an inhibitor of P450-based metabolism, rotenone was considerably more toxic than in untreated fish. This demonstrates that the status of the P450 system, as affected by exposure to other compounds, can affect toxicity in a subsequent exposure.

This is one justification for looking at this suite of enzymes in the context of environmental risk assessment. A population that has elevated P450 enzymes that are responsible for metabolic activation may be at increased risk to subsequent exposure. This might interface to uncertainty in a risk assessment.

Dr. John Stegeman and coworkers at the Woods Hole Oceanographic Institution (Stegeman et al., 1986) were able to detect significant increases in the major aromatic hydrocarbon-inducible form of P450 using an antibody probe, demonstrated in a western blot. One band, representing the elevated level of this protein in a deep-sea fish collected off the continental shelf east of New York, was contrasted with another band from a fish collected off the continental shelf

east of Nova Scotia. Dr. John Farrington's laboratory analyzed the livers from these fish and found that the levels of PCBs were much higher in the fish collected off New York. While we cannot say conclusively that these are causally related, the association is strong circumstantial evidence for a biochemical impact of pollution even in the deep sea.

There have recently been several field-oriented workshops on biological effects measurements. I would like to use some of the data that was generated during an International Oceanographic Commission (IOC)-sponsored workshop conducted by its Group of Experts on the Effects of Pollutants (GEEP) two years ago in Norway. In this study, investigators examined animals collected along a pollution gradient moving up a Norwegian fjord (Bayne et al., 1988). The practical workshop and the collection of papers that resulted from it represents a good example of the kind of study necessary to place subcellular measures in context with physiological and ecological measures. A comparison of the changes in cytochrome P450 monoxygenase enzymes in flatfish sampled from different stations along the pollution gradient showed a strong relationship between enzyme activity and the degree of pollution with aromatic hydrocarbons. This was compared with scope-for-growth measurements that were made by another group for the mussel Mytilus edulis. The scope for growth in the mussels decreased with increasing pollution just as the P450s in the flatfishes increased. This type of association gives us confidence that subcellular measures can be interpreted at higher levels.

An example of the relationship between subcellular measures and reproduction can be found in work done in the laboratory of Dr. Robert Spies at the Lawrence Livermore Laboratory in California (Spies et al., 1988a, 1988b). His group has been studying the starry flounder in San Francisco Bay and examining the relationship between cytochrome P450 activity in spawning females and various parameters associated with the viability of the young. The correlation between embryological success and P450 activity is significant, but not particularly predictive. These kinds of data are also provocative when attempts are made to put subcellular measures in context with ecological measures.

A promising measure which has received attention is the level of metallothioneins. These proteins are intimately involved in trace metal homeostasis and in the metal balance of the cell. They can in fact be protective. Studies have shown that when metal-binding proteins have been induced in animals, subsequent exposure often results in a lowered sensitivity. In other words, naive animals have greater sensitivity than those exposed to metals. Thus, the presence of high levels of metallothioneins in animals collected in the wild may be a signal of trace metal exposure.

Immune alterations. Another intuitively attractive measure is the functional status of the immune system. Measures of the functional status of the immune system and its compromise by chemical exposure have been highlighted as one of the important areas of suborganismal response that is poorly understood but potentially very important. The presence and spread of diseases like Dermo and MSX in shellfish stocks of the Bay have heightened this awareness in this region.

There are two types of measures that have been suggested for looking at immune suppression. For a direct measure, an organism can be challenged with a pathogen and the clearance of the pathogen followed. For an indirect measure, you can observe the functional aspects of the immune system: antibody production, phagocytic cell function, macrophage aggregation, etc.

Stress protein induction. Stress proteins are found in species from microbe to man and are rapidly synthesized in response to acute stress. Examples of these kinds of stress are heat (hence the original name heat-

shock proteins), oxidizing agents, heavy metals, steroid hormones, anoxia, low pH, viral infections, wounding, antibiotics, etc. Studies conducted by Dr. Brian Bradley on bivalves and copepods here in the Chesapeake Bay show how the various molecular weight proteins that are induced can be detected using immunological probes. It has been shown that the profile of the various heat-shock proteins induced may provide information regarding the particular form of "stress" incurred.

DNA alterations and genotoxicity. DNA alterations/-genotoxicity show great promise in revealing underlying factors in environmental carcinogenesis. They have been used largely in the context of PAH contamination and PAH-related lesions. As I stated earlier, PAHs are metabolized to reactive intermediates by the P450 system, a factor which may be related to the presence of cancerous lesions in areas heavily polluted with PAH. In general, this approach looks at changes in genetic structure, either in chromosomal structure at different phases of the cell cycle or in adducts that have formed from reactive species (e.g., the PAH metabolites).

Oncogene induction is one of the least explored realms. This is a very active area of mammalian cancer research. The approach is to look at the inappropriate or unregulated expression of genes that control cellular growth processes. It appears as though these phenomena may play a major role in the growth and proliferation of certain kinds of tumors. While investigation of these in the context of tumors in feral fishes is an important endeavor, it is unclear whether they will have widespread application in other forms of environmental testing. Studies on the molecular expression of other growth factors, such as growth hormone and thyroid hormones, however, may prove to have applications.

The disposition of those of us concerned with biochemical endpoints is that we hope to make measurements with integrative and predictive power. We aren't there yet, but some of these kinds of measurements have promise and utility.

Most of these measures haven't been looked at in standard toxicity testing protocols, but there is no particular reason why they couldn't be. Many of these measures could simply be added to the list of measurements taken at the end of the test. The problem is that people trained in one type of testing aren't usually trained in the other. Some coming together is needed.

On the subject of coming together, I want to explain why the decision was made to cancel the workgroup session on suborganismal reponses. This week in Keystone, Colorado, SETAC, the Society of Environmental Toxicology and Chemistry, is sponsoring a workshop on biomarkers. Many of the people we had hoped to have participate in the session here are going to be at this meeting. I have the list of participants if anyone is interested in seeing it. The format of that meeting is to have seven working groups, six in various topic areas and one synthesis group. There is a group titled Physiology/Other being chaired by Dr. Foster Mayer of the U.S. EPA; a group on Metabolites being chaired by Dr. Mark Melancon, now with the U.S. Fish and Wildlife Service in Patuxent; a group on Histopathology being chaired by Dr. Dave Hinton of the University of California-Davis; a group addressing Protein, Enzyme Induction/Inhibition being chaired by Dr. John Stegeman of the Woods Hole Oceanographic Institution; a group addressing Immunology being chaired by Dr. Beverly Ann Weeks of VIMS; and a group addressing DNA Alterations being chaired by Dr. Lee Shugart of the Oak Ridge National Laboratory. Those of you familiar with this field will recognize that a number of these people are the experts and it was felt that the results and recommendations put forth from this workshop would be directly applicable to the Chesapeake Bay workplan. I will make every effort to obtain and incorporate the results of the SETAC workshop in a timely fashion.

Questions

Q: Do you feel that in the regulatory context any biomarker should be tied in some direct fashion to growth, survival, or reproduction of an organism?

A: Not until we can show that link. With some of these endpoints, if we can show direct links to growth, we may have some scientific power and credibility. It will take a brave regulator at some point to regulate on the basis of these, and it will get battled out in court -- if it ever happens.

Q: EPA has been bludgeoned when it tried to use this kind of endpoint without proper basis. We need concepts that are usable and understandable by laymen and judges. The tie must be firm and pronounced.

A: You're right, it won't be easy.

Comment: Uses for biomarkers are various. They can be used, for instance, as an indicator of exposure with preliminary biological results. We have to know how we're using these and take advantage of them where we can.

Q: Extrapolation across levels of organization has been discussed. The other problem is extrapolating across species. In a study of San Joaquin kit foxes, we were doing blood samples for standard veterinary blood diagnostics. The response from the veterinary consultant looking at the samples was that if they were dogs they were dead. How do you deal with that kind of problem, when you can't develop for every species the kind of database we have for humans?

A: Yes, it's a problem sometimes even in the same phyla. For example, in a cruise on the Elizabeth River, we targeted toadfish for cytochrome P450 studies. Toadfish carrying high levels of PAH metabolites in bile showed no change in cytochrome P450-mediated enzyme activity, in tests done at three labs. In spot, responses were what you'd expect. Choice of species is crucial.

Q: Has anyone looked at Fundulus in chronically stressed areas vs. nearby unstressed areas?

A: Certainly. This type of study is traditional. Also, it seems that certain genotypes appear to be selected for in these situations.

Q: What about the theory recently expressed in Nature that extrapolation from rats to humans is absurd?

A: We can't test humans; we have to have surrogates, so even if they aren't perfect these studies have some value. At biochemical levels there are problems of extrapolation between species. In terms of toxicity, i.e., do they live or die, you also see inter-species differences, and these are often related to the same biochemical differences.

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Final Plenary Session

Concluding Remarks and Workgroup Session Reports

Moderator: Joseph Mihursky

Opening Comments

Joseph Mihursky

I'd like to remind you that support for this workshop came from the NOAA Coastal Resources Management Division through the Maryland Department of Natural Resources (DNR). This was a combined effort of Pennsylvania, Maryland, and Virginia. Jerry Hollowell (Susquehanna River Basin Commission), Laura Lower (Virginia Council on the Environment), and Cynthia Stenger (Maryland DNR) were involved in the planning effort. Dave Pyoas and Steve Jordan were key people helping in DNR. Close to two dozen people have contributed their efforts as chairmen, speakers, conveners, and staff. And of course the participation of so many people with so little lead time has been a gratifying response.

Let us consider the long view. An attempt at Bay restoration is in place. We have acknowledged problems and need to try to turn off the faucets, and explore pretreatment. Some information is needed on priorities, and we need to monitor what's there over time to discover what gives us the problems. Management or regulatory initiatives must be tracked in biological systems. There are already programs in place for water quality, phytoplankton, microzooplankton, mesoplankton (not all species), and benthic organisms (quantitative, population). A pilot effort is also in place to assess fishery stocks using conventional as well as acoustic techniques. This is the background against which we will begin to deal with toxics. It's not necessarily a regulatory, litigative situation. Over time you want to know if you see a system response in terms of stressors, species diversity, etc., resulting from a regulatory track. This workshop is a first step toward a framework of recommended best approaches.

A document from the workshop will be available to agencies. The workplan that will utilize the workshop document will be sent to the Monitoring Committee for input. Subsequent RFP's will reflect the funding levels set by September. The funding levels will determine the temporal and spatial coverage achievable. That is out of our hands. But we can offer our best science.

Risk Assessment Workgroup Report

Ian Hartwell

Our group discussed a variety of factors influencing how you do risk assessment in a diverse, complex system or in a pilot study, and how to formulate hypotheses. We asked whether and how we can apply risk assessment techniques to studies like this to answer the questions being asked. As a result, we developed an initial ranking scheme as recommendations to other groups, including ideas for how to combine the recommendations in ways that are valid to address questions. Analyzing the relationships to various factors responsive to the tests is recommended. The framework is done; details are lacking. Our ranking scheme is designed to help future efforts -- long-term monitoring plans. We still need to identify areas needing work.

A ranking system of five parameters was devised. The parameters are:

Consistency of results

Severity of endpoint

Degree of response

Number of tests

Reproducibility of results

Consistency refers to the agreement between the different bioassays the other workgroups are devising. For example, for a given test endpoint, what proportion of the species tested showed a positive result. For a given species, what proportion of the tests show a measurable effect. If the results from all tests and/or species agree, consistency is high, and confidence in predicting toxic effect (or lack of effect) is high. If only half the test results are positive, consistency is low. Thus the lower internal value for consistency is 50%.

Severity refers to the degree of effect which the bioassay endpoints measure. Mortality is the most severe response. Impaired reproduction is second, and impaired growth is third. Other endpoints can be included in the lists.

Degree of response is a measure of the proportion of organisms responding in each test. Unlike traditional methods of reporting toxicity bioassay results (e.g., LC50), risk assessment and hazard assessment utilize all response data. Thus a response level of only 10% is as important to know as the 50% level.

Both severity and degree of response are straightforward parameters if only one type of bioassay is run at each site. However, we anticipate that the other workgroups will propose a suite of tests to be run at different physical-chemical environments throughout the Bay system. Thus some system of factoring in the relative sensitivity of different tests and different tests species will be necessary to arrive at a ranking value for these two parameters after the other workgroups have made their recommendations.

The number of tests run at each site should be the same for statistical and experimental reasons. However, given the uncertainties of field work, this may not always be possible. The suggestion was made that if some sites don't have the same number of tests, the equivalent tests at other site should be discarded. Consensus could not be reached on the point but most participants did not recommend throwing out good data.

Rank of these criteria. (The group voted to weight the factors: 1=low, 10=high.)

Consistency	5.8
Severity	8.5
Degree of response	7.9
Number of tests	4.8
Reproducibility	6.6

Other discussions of the group evolved into the following list of further recommendations:

Define hypothesis specifically. What do you hope to learn?

Refine experimental design. Locate where you know you can show problems, and use one such spot as a test site to demonstrate that your methods work and to test the ranking system to see if it works.

Chemical cause-effect studies. What sorts of chemical contamination have which effects, i.e., which effects are most important to look at it?

Obtain an analytical hierarchy process. There is a software program for this, and consensus is that it's excellent for this use.

Sample sediment and water together -- in time and space.

Include temporal and spatial variation. Multiple samples and few sites is better than many sites and few samples.

Endpoints should address population parameters -- i.e., subtler effects.

Do water and sediment analyses at the same point. To predict in a probabilistic sense you have to have data on the species.

Develop modeling techniques to link bioassay and population impacts.

Create a database on relative sensitivity of assay species and endpoints. This recommendation bears on the debates about consistency, severity, and degree of response.

Fill out internal items of ranking factors after assay methods are chosen.

Questions

Q: This workgroup's primary job would seem to have concerned carrying out the recommendation to develop modeling techniques. Can you give any specific suggestions for such techniques?

A: A variety of modeling approaches are available to link bioassays with pollution impacts. They are not universally applicable or agreed upon. This is a generic recommendation -- there was not enough time at the workshop to come up with a tight model linking these things. This will have to be a long-term effort. As you will remember, we don't yet have the dose-response data that the risk assessment models usually use. This problem hasn't been approached by risk modelers yet.

Q: Are there no ecological risk assessment models for terrestrial ecosystems either?

A: The only exception I can think of is models of effects of gaseous pollutants on forests.

Q: Could you substitute percent-dilution data for dose-response and apply this method to aquatic systems?

A: Yes, except we aren't going to have dilution data.

Whole Organism Workgroup Report

Steve Schimmel

Our group tried to answer the questions in the workgroup charge (see questions at beginning of this workgroup's Discussion Notes in Appendix A). We considered the first question the primary one. To answer it, we developed a table of methods -- species, duration, and type of test to be used. We were conservative in our approach, basing our draft recommendations on tests already established and accepted, such as NPDES permits and pesticide registration. Methodologies that have not proved workable were not included.

You will also notice from the chart that second-level tests are not as precise, but include species important to the Bay. Third-level tests basically remain in the research realm, but may in the future be of use.

By answering question 1, we also essentially answered numbers 2-5.

We gave some attention to suborganismal measures (question 6). Our consensus position was that if we can reasonably incorporate these measures, we should. The major question is on costs. Conceptually and philosophically we think suborganismal tests should be included. The managerial reaction is questionable.

But the future of aquatic toxicology will probably rest with these suborganismal tests. We recognized that some biomarkers are best used not with toxicity testing but with field collection of indigenous organisms, in tandem with pathology. The estimated causes and effects once you see toxicity are where these can be pertinent.

For the next question (7), we did not work much on the analytical chemistry associated with these tests. Toxicity data should drive analysis as much as possible. That is the economic reality. First you find an effect, then you look to analysis to explain it.

As far as the risk assessment discussion, we reached no new conclusions. The top tier of tests gives population responses that might be needed. There is not much that gives "r," or time to reproduction -- only *Ceriodaphnia.*

The tests discussed here apply to laboratory situations -- either static or mobile. We did discuss *in situ* testing, but there's not enough data to be able to discuss it except as research.

The whole organism group agreed with the sediment group that there should be a control Chesapeake Bay site. Questions about controls are not resolved.

Questions

Comment: The striped bass larval test is useful only in the lower end of the salinity range of the estuarine cell -- they can't survive in 25 ppt water.

Comment: Biomarkers people would adopt an additional strategy. Adding biomarkers into acute and chronic testing makes sense. At the same time, there is a need to incorporate this approach into traditional environmental living resources monitoring. We can gain a lot of information by looking at indigenous organisms, either at the biochemical or biological level. I will try to draft a document on how this fits into our overall goals, circulate it, and aim for producing a position statement.

Comment: Maryland DNR thought about this some years ago. They did a bit of it, but it was terminated for lack of funding.

Whole Organism Tests for Ambient Toxicity in Freshwater, Estuarine, and Marine Environments of the Chesapeake Bay

Category	Freshwater environment	Estuarine environment (max. 25 ppt)	Marine environment
Proven Regulatory Methods	*Ceriodaphnia* 7-day chronic Fathead minnow 7-day chronic and embryo/larval test *Selenastrum* 96-hr	Sheepshead minnow 7-day chronic and embryo/larval test *Menidia beryllina* 7-day chronic *Mysidopsis bahia* 7-day chronic Bivalve larvae: *Crassostrea virginica* *Mya arenaria* *Mercenaria mercenaria* *Skeletonema* algal test 48 hr?	Sea urchin fertilization test *Menidia beryllina* 7 -day chronic Sheepshead minnow 7-day chronic *Mysidopsis bahia* 7-day chronic Bivalve larvae test: *Mytilus edulis* *Champia parvula* reproductive
Established Methods Pertinent to Chesapeake Bay	Embryo larval: bluegill, catfish Striped bass larval toxicity test	Grass shrimp larval acute lethality, 96 hr Striped bass larval toxicity test (low salinity)	Grass shrimp larval acute lethality, 96 hr Striped bass juvenile 96-hr acute lethality
Research Methods Pertinent to Chesapeake Bay	Duckweed 96-hr	*Anchoa mitchilli* 96-hr test *Calliglossa* algal chronic Sago pondweed toxicity test *Eurytemora affinis* *Neomysis americana* 96-hr acute *Acartia tonsa*	*Acartia tonsa* 7-9 days *Anchoa mitchilli* 96-hr acute lethality (larval)

Sediments Workgroup Report

Richard Peddicord

Sediment toxicity tests can be very useful for some purposes. Major points from our discussions are summarized below.

Sediment quality criteria -- Even though they are still under development, we should collect the parameters necessary to calculate compliance, insofar as we know what the parameters are.

There is a powerful consensus that we need to pull together all relevant information on toxics in Chesapeake Bay before we design field operations. Then we can determine what's appropriate to do, where, whether emphasis should be on the water column or sediments. All this should precede design of field sampling.

Most of the off-the-shelf sediment toxicity tests are adaptations from water tests. We have a need for tests aimed specifically at sediments and population endpoints. Short-term mortality of adults or juveniles is the most common endpoint among presently available sediment tests. We need to see more tests addressing the next generation.

Sampling for toxicity testing should include some sites expected to give very strong response. We can work across a gradient to see how well toxicity tests correspond to our conception of what's there, and see what kind of sensitivity is present. Does a zero result indicate low contamination, low bioavailability, or low sensitivity?

In surveying the Bay for degree of degradation, it is important to look not just at sediment toxicity, but also at benthic community information and sediment chemistry information.

There are two types of exposure methods: those using whole sediments and those using extracts of sediments. Tests using pore water and organic extracts of sediments are viewed skeptically. If we apply tests in the near-term, we must use tests and species with well-established databases. We need to anticipate issues of lab maintenance or test organisms and variability in response.

We need to think in terms of a suite of different kinds of toxicity tests -- different species, different tests. As a rule of thumb, amphipods are among the more sensitive species, and we have concentrated on them. However, we still need other species in which there is an indication of relative sensitivity.

In developing tests, we should emphasize some indigenous Chesapeake Bay species, with at least some endpoints that address the next generation's success.

It is extremely important to develop protocols related to the specific objectives of this program, standard operating procedures, and quality control/quality assurance measures before getting very far into the studies.

Studies of bioaccumulation could consume great quantities of time and money, and should not be part of an initial survey. Where problems seem likely, we could look at potential linkages between sediment and tissue contaminants. This would provide a context for interpretation. These techniques should be used in the framework of a hypothesized problem (such as contamination in fish for human consumption), not in a survey situation.

Work on biomarkers should be encouraged, but they are not very useful at present. They will become more useful when links have been developed between biomarkers and whole organism responses.

The final point in risk assessment is relevant to the entire effort in ambient toxicity assessment: At the program level and below, we need carefully defined objectives in the form of testable hypotheses. We must clearly state exactly what we are trying to determine.

Questions

Q: Has any attention been given to resuspended sediments as opposed to those in place?

A: No -- our group didn't work that broadly. Resuspension of sediments is important in terms of contaminant bioavailability.

Q: Was fluidized mud addressed?

A: No.

A closing observation concerns appropriate controls. We talked about controls and reference sediments. The relative importance of that question may be dependent on the objectives. If you're doing a survey of intensity of response, it may not be very important. For hypothesis testing, it is crucial. Thus selection of appropriate reference points involves managerial as well as technical issues: do you want a comparison with pre-John Smith sediments? or with the cleanest you can find now?

BIOASSAYS FOR SEDIMENT TOXICITY TESTING IN FRESHWATER, BRACKISH SALTWATER, AND HIGH-SALINITY ENVIRONMENTS IN THE CHESAPEAKE BAY

Organism	1 Availability	2 Taxonomic/geographic relationship to site inhabitants	3 Toxicological database	4 Demonstrated sensitivity	5 Ease of laboratory maintenance	6 Ease of test method	7 Ecological/economic relevance	8 Insensitivity to geophysical factors	9 Level of sediment exposure	10 Route of exposure	11 Test endpoints	12	Notes
Standard bioassays													
Freshwater													
Hyallela	X	X		X	X	X	X	X		So		L	
Chironomus	X	X	X							So		L	
Hexagenia		X	X	X		X	X		X			L	
Brackish saltwater (0-15 ppt)													
Hyallela										So		L	
Eohaustorius		?			X	X	X	X	X	So		L	
High salinity													
Rhepoxinius	X		X	X	X	X	X		X	So	a,b	L	
Ampelisca	X	X	X	X	X	X	X		X	So	a,b	L	
Bivalve larvae 48-hr pediveliger oyster *Mercenaria*	X	X	X	X	X	X	X			SS So	a,c a,d	 L	
Polychaete *Neanthes (Nereis)*		X	X		X	X		X	X	So		M	
Mysid (grass shrimp)	X	X	X		X	X	X	X	X			M	
Bioassays available based on enhancement of standard techniques													
Low salinity													
Leptocheirus	X	X		?	X	X	X	X	X	So		M	
Eohaustorius										So		M	
High salinity													
Lepidactylus	X	X	?	?	X	X	X	X	X	So		M	
Bioassay techniques under development or recommended for development													
Chronic tests													
Leptocheirus										So		H	
Lepidactylus										So		H	
Sago pondweed	X	X			X	X	X		X	So		H	

(10) So=solid phase; SS=sediment slurry

(11) a=acute lethal; b=behavioral effect; c=abnormal development; d=metamorphic failure, etc.

(12) L=low cost; M=moderate cost; H=high cost.

General Perspectives on the Role of Biomarkers (Biochemical Measures of Effects) in the Chesapeake Bay Toxics Workplan

(Comments compiled from Dr. Ken Jenkins, Dr. Brian Bradley, Dr. Guri Roesijadi, Dr. Margaret James, Dr. John Pritchard, Dr. Wolfgang Vogelbein, and Dr. Dave Wright, and submitted by Dr. Jay W. Gooch, suborganismal plenary session speaker and convener)

Although the suborganismal responses workgroup session was not formally convened at this workshop, a number of investigators with research experience in this field were present. This document has been prepared in collaboration with those present and is intended to reflect other important comments and/or suggestions related to the use of biochemical effects measurements for the assessment of ambient toxicity in Chesapeake Bay waters.

The general consensus of the workgroups was that biomarkers were clearly the tools of the future, but concern was expressed that they may not yet be in a form where they can be used on a routine basis. We propose, however, that subcellular biomarkers (biochemical effects measurements) can be used effectively in conjunction with the conventional bioassay studies that were the major topic of the workshop. Carrying out a range of biomarker tests in conjunction with the proposed bioassay program would provide a number of important advantages to the Chesapeake Bay Toxics Program:

> Subcellular biomarkers are substantially more sensitive to toxins than conventional bioassay endpoints, and their implementation in this program would provide a more accurate picture of the distributions of low-level toxins in the Bay.
>
> Unlike conventional bioassay endpoints, subcellular biomarkers can provide information on the types of pollutants responsible for any observed toxicity.
>
> Carrying out subcellular biomarker tests in conjunction with well-characterized whole-organism bioassays will allow these new methods to be calibrated and validated in a well-defined and experimental framework. These tests will provide a basis for rigorously defining the
>
> relationship between subcellular endpoints and parameters such as growth and reproduction.
>
> This provides a cost-effective approach to optimize the information obtained from the toxics program.

We propose that subcellular biomarkers can also be used effectively with ongoing field survey programs. In these studies, biomarker assays can be performed on native organisms and the results compared with both chemical and biological data, which are normal components of these programs. We recommend that indicators of contaminant-induced changes be measured in conjunction with ongoing sampling programs being conducted in the Bay. The numerous sampling efforts aimed at the monitoring or evaluation of populations of Bay species could accommodate these studies at more modest cost than initiation of a new program. As significant resources are already devoted to the task of sampling resident species, it appears economically wise to extract as much information as possible from the effort. Again, this approach would provide information on sensitive sublethal effects in native organisms and provide insights into the causative agents when toxicity is observed. It would also allow biomarker endpoints to be correlated with both chemical and biological field data to further calibrate and validate these procedures.

In his plenary presentation, Mr. Steve Schimmel of the EPA's Narragansett Laboratory suggested that

acute toxicity bioassays conducted on ambient waters of the Bay would almost certainly be negative, as has been the case for most of the ambient toxicity surveys conducted by the EPA. That is, experience suggests that acute toxicity problems remote from known sources of pollutants are very rare. In fact, chronic effects, as defined by bioassay protocols, are also somewhat rare. Despite this, many coastal waters like those of the Chesapeake Bay are experiencing population declines of ecologically or recreationally important species. It is widely perceived that at least some of this decline may be due to pollutants. Biomarkers may provide the mechanism to detect consistent, repeatable measures of chemical stress in indigenous biota.

Bay Programs which may be able to incorporate pollution studies:

- State finfish and shellfish contaminant monitoring programs
- Benthic infaunal surveys
- Fish and shellfish population surveys (stock assessment)

  Habitat surveys
- Field-oriented research programs (Sea Grant projects, etc.)

Used in the appropriate context and with the appropriate questions in mind, incorporation of biochemical effects measurements into ongoing sampling programs in the Bay should provide important additional information regarding the *effects* of toxic chemical contaminants.

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Appendix A

Workgroup Questions and Discussion Notes

The workgroup sessions were instructed to address specific questions on the topics. These questions are attached to each workgroup's discussion notes. The highlights and points of consensus for each workgroup were synthesized and presented in the final plenary workgroup reports.

Questions Related to Population Risk Assessment

The objective of the Risk Assessment Workgroup is to develop a risk assessment scheme (preferably quantitative) which can be applied to the whole Chesapeake Bay or its subsystems, for the purpose of risk management at the state and federal government level. Secondary uses of the scheme are to guide monitoring strategies and provide damage assessments. In arriving at such a scheme, the following questions and subsidiary considerations should be addressed.

I. Technical questions

A. What is the mathematical approach?
   1. qualitative
   2. quantitative
   3. other

B. How to compartmentalize the model to accommodate input?
   1. whole organism toxicity
   2. sub-organismal toxicology
   3. sediment toxicity
   4. physiographic regions
   5. specific sites

C. How to estimate chemical exposure?
   1. water
   2. sediment
   3. atmospheric
   4. discharges
   5. fate and transport
   6. other

D. How to define receptors and endpoints?
   1. field biomonitoring
   2. lab studies
   3. surrogate/indicator species
   4. acute toxicity
   5. sublethal responses
   6. population/community responses
   7. other

E. How to incorporate temporal variations?

F. How to calculate uncertainty?

G. How to field verify?

H. What are the model outputs?
   1. risk predictions
   2. monitoring guidance
   3. research guidance

II. Practical questions

A. Has the approach been used elsewhere (does it work)?

B. What are the operational requirements?
   1. level of personnel expertise
   2. time commitments
   3. computer facilities
   4. cost

C. What are the data requirements?
   1. what kind of data
   2. how much data

Workgroup Sessions on Population Risk Assessment

Convener and Chair: Ian Hartwell

Most of the research of the Chesapeake Bay Program has been focused on nutrients, not toxics. The 1987 Chesapeake Bay Agreement has various objectives for Bay restoration, including a Toxics Reduction Strategy, among other items. Our objective at this meeting is to provide scientific comments on the strategy and the pilot biomonitoring study.

One of the goals of the strategy is to produce quantitative risk assessment on commercially, recreationally, and ecologically important species, and on the system itself.

The Basinwide Toxics Reduction Strategy states that information generated from the pilot phase assessment will be used to estimate a risk. No assumptions will be made about chemical exposure; rather, biological effects will be used to assess the need for further study.

The workgroup did not agree with this view. Risk assessments cannot be created in the absence of chemical exposure information.

What do the states want?

Steve Jordan (Maryland Department of Natural Resources): The Bay Agreement of 1987 has as its primary goal to protect the Bay's living resources. We have nutrient goals that we think will help and other ways of improving water quality. The toxics area is the weakest. The Basinwide Toxics Reduction Strategy is more specific. Commitments include a toxics loading inventory. We want to use bioassays to estimate risks to populations of living resources. The key question is how to extrapolate to real populations. It is the populations that managers care about, not individuals. Managers need to know whether an important population is at some risk, and they need to know the magnitude of risk and the confidence level of our prediction.

The Virginia participants agree with Jordan.

Question: Do we want to develop risk assessment or damage assessment?

These items were discussed as distinct entities. The first is a predictive assessment while the latter is retrospective in nature. The effects of toxics as opposed to confounding parameters such as acid rain, anoxia, turbidity, overfishing, etc., were discussed. These items must be taken into consideration when interpreting the field bioassay results. It was decided, in light of the proposed workplan, to adopt a tiered approach.

Comment: We must be careful when demonstrating cause and effect. The mechanism may not be known, so we should be cautious in pursuing these mechanisms, being wary of confounding factors.

We must also address techniques that can incorporate mixtures, since we're really looking at a toxic soup. That is the purpose of sublethal biological assays. They allow us to examine whether inputs like acid

rain and pesticide runoff are causing problems in addition to those caused by effluents.

Identifying these problems should be the first step addressed in the field. Correlating the issues of cause and effect is the next problem. Where you have contaminants you're likely to have high organic loading. We will need to differentiate the toxic effects from the effects of organic loading. But first we will demonstrate that there is a problem, and then demonstrate the cause/effect relationship. Then the third step is to look at what is in the mixture so we can concentrate on that particular chemical or series of chemicals. This is analogous to the NPDES permitting process. The permittee analyzes the effluent and then must figure out what part of the effluent needs to be cleaned up. They reconstruct the effluent or fractionate it in order to find the culprit. We will need to do the same thing.

Point: Those are management questions. We need to focus on an assessment system that will allow the risk managers to find the culprit and the organisms at risk. Risk assessment also needs to address other factors like anoxia.

Response: We need a tiered system that asks, "Do we or don't we see problems?" We need a tool to look at stresses; and if we find them, then look deeper. The next question is how to manage that. We have to ask whether it's toxics-related, or an effect of habitat or another impact like overfishing. Then we ask, "Is it a pollutant? What type of pollutant is it?" Fractionation doesn't solve the problem because it may change the overall toxicity.

Comment: We will be working in the context of information that is already fairly well described. With a tiered approach, we've done the first tier; we know that there is a problem and that it's not totally pollution-related. There are other problems such as overfishing. To address the toxics, we should focus on mortality, growth and development, and reproduction of organisms. These are the critical factors for maintaining a population. These can be used as categories, and other factors can be related to them. Assays should reflect these categories. Otherwise there will be problems.

Question: Will we collect chemical data?

According to the proposed workplan, sediment and water chemistry data will not initially be available. The group strongly urged that at least routine water quality data (i.e., O_2, salinity, pH, turbidity, etc.) be gathered at each test site. Also, any biological tests performed should be selected such that the data can address or be capable of being extrapolated to community effects. In the absence of chemical data, loading estimates at a known contamination site could be used to exposure estimates in a risk assessment. The option of creating a risk assessment vs. a damage assessment was revisited.

Response: That type of data collection will be driven by the results of the first biological analysis.

Response: The draft workplan suggests that we begin with field assays and apply chemical analysis only where we get positive results, and preferably for implicated chemicals. There is already a lot of body burden information but it does not tell us anything about population level responses.

Comment: You should definitely collect general water quality parameters at the same time, to eliminate other causes such as DO.

Response: These systems are already well characterized for this.

Point: We should come up with a rational system to take field bioassay data and find out if it's telling us that toxicity is a problem. Risk assessment involves exposure assessment, which involves field observations -- biomonitoring, etc. Pre-supposing that you

know the toxicity, you have the exposure component and the toxicity.

Response: Yes, but we don't have input on exposure assessment, so should we do risk assessment or damage assessment?

Question: We will have information on growth, mortality, and reproduction; what will we do with this?

Response: Damage assessment is taking stock of what has occurred. It is retrospective, as opposed to risk assessment, which is predictive. Also, there is hazard assessment, which is the inherent danger of chemicals.

Question: The state wants a method that has been used and is known to work. Is there such a model or approach already developed that could use this information?

Question: Should we be looking at risk to populations, or are we interested in what will be expected to happen to the community?

Response: It could be both. For both populations and communities the information acquired must encompass the representative trophic levels.

The workgroup discussed the types of data required. For assessment purposes dose response data or response surface calculations are more useful than LC50 or EC50 data. Also sublethal endpoints are better than lethality tests.

Point: We should divide the Bay into geographical areas and then look at the hydrological, biological, and ecological effects. Also, we must assess the cost and prioritize what we really want to do.

Question: What can we do with the pilot scale, and then what do we want from it in order to interpret the Bay in the long run?

Response: We need to proceed with a qualitative system, ranking information from pilot studies, and we need to be able to expand it.

Response: No, I think we will have to get qualitative **and** quantitative data depending on the problem we are addressing. For example, if a chemical is a carcinogen, we don't even have to subject it to other tests.

Comment: This won't happen. We will not have chemical data up front.

Response: But it can, and other tests must appropriately assess hydrological, geological, biological, and ecological factors. We have to figure out what our strategy is.

Comment: It is important to let the other workgroups here know whether we want hypothesis testing or continuous data.

Point: A comprehensive toxics database for the Bay does not exist. The primary need of the states is to determine how to handle monitoring data; that is, sublethal toxicity data. Are there toxics problems outside of hot spots? And how can we best answer those questions? What is the best way to handle the data?

Response: We need to stop presenting results of tests in terms of statistical differences. Results may vary greatly. So we should tell the toxicologists to express results in terms of magnitude. We need data more like dose-response; not pair-wise comparison. How will we infer causation from those kinds of data? There are potential mistakes, like inferring toxicity when you're really seeing differences caused

by physical factors. Also, to catch episodic events their strategy must be designed appropriately.

The workgroup discussed in more detail the importance of specific endpoints in light of the proposed workplan limitations and the tiered assessment approach. The biological tests should be related to population effects. Field studies are more relevant but may be difficult to interpret relative to toxics effects. Toxicological studies are more straightforward but results must be extrapolated to the field. Validation studies are required.

Question: OK, but what do we tell the state? I suggest we do it in terms of a tiered approach.

Response: I'll design a proposal to tell the state where we are, which is essentially a statement of need. It can provide a workplan with various tasks, with a number of scenarios, a schedule, and an estimate of how much it will cost. We should write a proposal and let them fill it in.

Comment: A lot of that exists. We should decide if it is good, and if not, modify it. This strategy is a draft and we do have input. We should decide if the tasks are adequate or not.

Point: Other groups are proposing that in addition to the Potomac, we should consider adding Baltimore Harbor and the Elizabeth River. Also, the Statement of Purpose calls for monitoring of effects on living resources. This is to learn effects, not to establish regulations or anything else.

Question: Take a scenario. If we see a strong gradient of toxic effects down the Potomac in the pilot study, how do we use that data to tell the state what they should do next?

Although this question was not answered, most participants felt that the study design was flawed.

Some participants feel that we need to define the endpoints so they are sure to be relevant to biological toxicity and effects on populations.

Question: Why don't they just do field studies?

Response: That can be very difficult. For example, Hall did a study of larval striped bass in the Nanticoke River, and mortality occurred in river spawning grounds, but not in the control (Vienna town water). This indicated that something in the water was the cause of the problem. This has since been thought to be low pH and high Al levels.

Point: We should address the package that the state has given us to find its limitations and a way to optimize this structure. Then we can tell them what they can get, and also what they can't get from the proposed structure.

Question: Let's assume the state has given us toxicity information for a fish, an invertebrate, and a phytoplankton species. Now what do we do?

Response: Give the state a system to rank the sites by degree of response. Produce a qualitative ranking system.

Comment: It's hard to differentiate the noise level from the effect of toxics. Theoretically, if mortality in striped bass from larvae to juvenile is normally 95%, an increase to 96% (which is probably not detectable) will halve the number of recruits.

Question: So what is the significance of the ranking system?

Response: What endpoint would we be most interested in, and what would bother you the most? Remember that direct toxicity to some organism implies indirect toxicity to other trophic levels.

Comment: They may have to develop a more sophisticated community level study like the MERL mesocosms.

Comment: From the Nanticoke example we can see that they didn't find the problem. If we knew why, it would be helpful to us.

Point: You can't rely on toxicity tests alone. You have to incorporate field studies. Beware of false-positive results, or false-negative results.

Comment: The SAV decline, thought to be due to herbicides, was decided to be due to shading, and now they say it may be herbicides again. In some areas the most sensitive lifestage --germination -- was not studied due to lack of ability to handle the seeds.

Comment: We need suborganismal tests for this reason.

There was some discussion of what we learned from our experience with the SAV decline and how to apply it to the ranking scheme.

The workgroup discussed how to characterize site-specific results in a ranking scheme to contrast different sites. Tests employing more than one species yield more information but require sensitivity calibration. Field effects may be due to direct toxicity or indirect community effects. The group strongly urged that sampling be done for both sediment and water at the same place and time, and that the draft workplan be modified to increase spatial and temporal replication. It was suggested that sites known to be contaminated be included in the effort. Also, the hypothesis to be tested in the monitoring study needs to be clearly defined and test endpoints should be selected accordingly.

Question: But what's going to be in the ranking system? We should provide the states with a ranking scheme so that they can do site characterizations.

Comment: We have done site characterizations, but they have been waste sites.

Question: Given a suite of sites, can we develop a preliminary methodology to determine whether there is a risk? That is, with some group of test species, what parameters would be good to look at?

Comment: It will be important to know the severity of the endpoints and to have consistent results. Also, we want the severity of effects to be measured in a graded fashion, as opposed to "yes or no" answers. And we should also use multiple types of species in consistent studies.

Question: Should a species be chosen that can tolerate a wide salinity range?

Point: We must consider the sensitivity of the test species.

Point: Inter-species variation can be handled statistically. However, we should decide whether we'll use the same suite of test species or whether that can be variable. For ranking, relative sensitivity will vary with the substances and other factors that are involved.

Point: There is a problem, though, because it may affect a parameter like competition which, in turn, may affect the endpoint.

Response: That's all right since the endpoint is still being affected by the toxicant, even though the effect may be indirect.

Point: They should do parallel testing of the sediments as well as the water.

Point: Many variations such as temporal variation need to be considered.

Point: You may want to add less severe endpoints so you can see low impact areas as well.

Question: But what if negative effects are a result of our ignorance, not of the lack of toxicity?

Response: We should have a strategy to choose stations.

Response: They do have a strategy as explained in the document.

Point: If they expect to draw a relationship between the effects and toxicants as a cause, they should choose sites where they have the maximum likelihood of that occurring.

Point: Don't overemphasize the number of locations tested at the expense of replication and observation over time. There may be variation due to time of year, weather, species present, and the like.

Point: Keep in mind that in an estuary, it's hard to establish a pollutant gradient system because you're moving in and out of many physico-chemical sub-gradients, which have different impacts on pollutant effects.

Point: All we can recommend is a meaningful way to form the sampling strategy, because we don't have a budget.

Point: Field tests should be carried out in an area where you know effects exist.

Comment: Information exists that the tests do work. It's the temporal and spatial effects and the like that would cause variability.

Comment: A previously-derived report may serve as an example here. This was an NRC panel specifically addressed to monitor the Chesapeake Bay, to determine how we could design a monitoring program. They had similar concerns on temporal and spatial processes.

Question: Are these types of sampling sites appropriate? necessary?

Response: If the hypothesis is that there is no measurable toxicity in the ambient waters or sediments in the Chesapeake Bay, you still need to get into severity and degree.

Point: We need water and sediment chemistry, but that can't be done right now.

A ranking system of five parameters was devised. The parameters are:

- *Consistency of results*
- *Severity of endpoint*
- *Degree of response*
- *Number of tests*
- *Reproducibility of results*

Consistency refers to the agreement between the different bioassays the other workgroups are devising. For example, for a given test endpoint, what proportion of the species tested showed a positive result. For a given species, what proportion of the tests show a measurable effect. If the results from all tests and/or species agree, consistency is high, and confidence in predicting toxic effect (or lack of effect) is high. If only half the test results are positive, consistency is low. Thus the lowest internal value for consistency is 50%.

Severity refers to the degree of effect which the bioaassay endpoints measure. Mortality is the most severe response. Impaired reporduction is second, and impaired growth is third. Other endpoints can be included in the lists.

Degree of response is a measure of the proportion of organisms responding in each test. Unlike traditional methods of reporting toxicity bioassay results (e.g., LC_{50}), risk assessment and hazard assessment utilize all response data. Thus a response level of only 10% is as important to know as the 50% level.

Both severity and degree of response are straightforward parameters if only one type of bioassay is run at each site. However, we anticipate that the other workgroups will propose a suite of tests to be run at different physical-chemical environments throughout the Bay system. Thus some system of factoring in the relative sensitivity of different tests and different tests species will be necessary to arrive at a ranking value for these two parameters after the other workgroups have made their recommendations.

The number of tests run at each site should be the same for statistical and experimental reasons. However, given the uncertainties of field work, this may not always be possible. The suggestion was made that if some sites don't have the same number of tests, the equivalent tests at other site should be discarded. Consensus could not be reached on the point but most participants did not recommend throwing out good data.

Reproducibility is the statistical measure of variability within a tests at a given site.

Candidate System

Comment: There is a software package by Thomas Saaty at Pitt. The name, AHP, stands for Analytical Hierarchy Process. It is not predictive, it simply takes qualitative evaluations and develops a ranking system. It can compare many factors and is relatively inexpensive.

Many of the committee members are familiar with this, and there seems to be general agreement that such a package would be helpful.

Question: Where are you measuring the uncertainty of effects?

Response: Under "degree of response."

Point: Anything that we come up with is also useful in other locations outside the Bay where similar environmental situations exist. This is important because of the lack of actual estuarine bioassays.

Question: Areas are chosen largely due to their importance to commercially important species. Should this be of concern?

Question: How do we utilize the endpoints from the other workgroups? Is transferability among sites and organisms important?

Response: If the information transfers to other areas, good. But that is not our task. However, if it doesn't transfer, we have to ask whether it is because the system we are working in is unique (due to size for example), or because the scheme we have proposed is not good. Transferability is the sign of a good scheme or theory.

Point: We should use a Chesapeake Bay organism. But we should be able to transfer to different systems. Can we address specificity to a given chemical? There is agreement that the protocol will not address specific chemicals, or even types of stressors, i.e., anoxia and nutrients.

Comment: We should assign weight to each factor.

Response: No, just use analytical hierarchy process, AHP. A group of experts should determine the ranking scheme for the AHP. This should be a suggestion of this committee.

Which of the ranking criteria are most important?

As an initial evaluation of the ranking factors, each member of the workgroup assigned a score to each factor to reflect its relative importance.

Ranks were tallied on a scale of 1-10, and results were:

Consistency	5.8
Severity	8.5
Degree of response	7.9
Number of tests	4.8
Reproducibility	6.6

These values could be used as weighting factors, but would require validation to use. The values here represent only the quick polling of a small group.

Questions to workgroup

I. Technical questions:

A. *The Mathematical approach* will be qualitative; however, input data is quantitative. The approach may become quantitative as the monitoring process develops.

Comment: You will have chemical data if you get a positive from the preliminary screening. We should do chemistry if the biology dictates it. If sample fractionation is used, you do have chemical information.

Comment: You should also do chemical analysis on samples that are not positive to see what is in the samples that are not toxic, and compare these to the toxic samples.

Comment: The chemistry should be done to validate the endpoints and species chosen, but it will be hard, due to cost.

Comment: Include chemicals that you think may be a problem. For example, look at the pesticide dimilin. We could find areas where it has not been sprayed to use as reference sites.

B. *Compartmentalization* of both sediment and whole organism toxicity will be measured by sublethal parameters, and if appropriate, so will any proposed suborganismal tests.

C. *Chemical exposure* estimates cannot be done directly as planned without chemical sampling. It could potentially be done later or with historical data if the site is known.

D. *Receptors and endpoints* can be defined after looking at field biomonitoring, sublethal responses, and surrogate indicator species. This will lead to the generation of population/community responses.

E. *Temporal variations:* The experimental design should address a gradient and a time factor. If you're not doing a time-course study, at least note the time of sampling for future reference.

F. *Uncertainty* is associated with spatial and temporal scales, and with sensitivity of species and test systems. The effective question is how to estimate probability. Information theory can be used in this context. Possibilities are the Analysis of Extrapolation Error approach and the Cluster Hypothesis.

G. *Field verification:* Use the ranking system to determine a set of sites to go back to and then see whether this area is hazardous by using a complete battery of tests. This will verify the method.

H. *Model outputs:* We don't provide a model; it's more like a system. It will guide monitoring and research. If the chosen site has a gradient, then the basis for choosing the gradient will be historical data. You can take samples and freeze them, but this may change the partitioning of chemicals between particu-

lates and water. You could definitely freeze assayed organisms for later suborganismal studies.

II. Practical questions

A. *Prior use.* Ranking approaches have been used and are accepted, but this particular set of factors has not been used, since it is specific to this monitoring program.

B. *Operational requirements.* The modeling links may require specialized people, and creating databases may take a lot of time. We may also need trained people for this. The group generally agrees that two or more person-years would be needed.

C. *Data requirements.* Data should be population-related. We need practical data, the more the better.

Question: Is there a firm toxics database? It seems that there may be some data, but it may be concentrated in hot spots such as Hart-Miller Island, Baltimore Harbor, and Elizabeth River.

Comment: PAH contamination comes from combustion products in the atmosphere.

Recommendations for the pilot study

- Define the hypothesis for the pilot and long-term monitoring study.
- The experimental design needs a great deal of refinement with particular emphasis on quantity and quality of test sensitivity.
- Do specific cause-and-effect chemical studies.
- Look into the Analytical Hierarchy Process as a ranking system integrator.
- Fill out internal items in the ranking factors, etc., after methods are chosen.
- Do sediment and water column tests at the same time and place.
- Coordinate sampling to consider temporal and spatial variation.
- Address population parameters using endpoints from biomonitoring tests.
- Do water and sediment chemical analysis at some point.
- Develop modeling to link field toxicity bioassays to population impacts.
- Create databases of the relative sensitivities of assay species.

Questions Related to Methodologies for Whole Organism Toxicity Testing

The whole organism toxicity testing session will have two plenary speakers. Each speaker will separately address "state of the art" techniques for either laboratory or field toxicity tests. The following questions should be addressed by the workgroup after these presentations.

1. What are the most appropriate laboratory and field toxicity tests for evaluating ambient toxicity in the water column of the Chesapeake Bay watershed?
2. Which laboratory and field tests are appropriate for the various types of habitats that need to be evaluated (i.e., streams, rivers, open Bay)?
3. What are the relative costs for each type of test?
4. What are the most appropriate test organisms that should be used for these tests?

5. What biological end-points should be used?

6. How can sub-organismal measures be incorporated into traditional acute or chronic toxicity tests? How can results be interpreted in terms of [risk to/impact on] living resources and their habitats?

7. What type of water quality and contaminants data should be collected during ambient laboratory and field toxicity tests?

8. Is additional research needed to develop laboratory and field methods for assessing ambient toxicity in the Chesapeake Bay watershed?

9. How can data generated from laboratory and field toxicity tests be used in "risk assessment"?

Workgroup Sessions on Whole Organism Methodologies

Convener: Lenwood Hall
Chair: Steve Schimmel

Hall: Workgroup participants should refer to the questions distributed previously and use them as an outline. The Maryland Department of Natural Resources is planning a 1-2 year pilot study of ambient toxicity, probably on the Potomac River, to determine the extent of ambient toxicity problems within the Chesapeake Bay basin. The results of this workshop are to be applied to that study.

Schimmel (US EPA): National methodologies exist for coastal environments and have been applied from Maine down to Florida and around the coast to Texas, with separate methodologies for the West Coast. My presentation yesterday described effluent and ambient tests representative of the Narragansett, Rhode Island Lab. These may or may not have direct pertinence for the Chesapeake Bay system. Perhaps the end-points are pertinent, even if the organisms used are not, but our purpose here is to develop a list of methodologies for the Chesapeake Bay. I am not partial to any one group of tests; we are working together to assemble a suite of tests that may be used here.

Purpose of the pilot project: to develop a suite of tests that are feasible, pertinent, and useful.

Locations of sites. Maryland is leaning toward using the Potomac River because it encompasses a variety of problems encountered in the Bay, including heavily polluted sources and freshwater areas. It was felt that exclusion of Baltimore Harbor and Elizabeth River was unwise, because if ambient toxicity testing will work at all, it will work at these sites.

Review of points 1-5 in the charge to the workplan, p.4:

1. biological responses to be measured
2. technology available
3. cost
4. sensitivity to sublethal toxicity
5. significant effects.

In considering the selection of freshwater/saltwater species for laboratory tests used in the pilot study, the lab tests should encompass: species pertinent for regulatory purposes, species not pertinent for regulatory purposes, and any additional alternatives suggested by the workgroup here.

A good starting point will be the NPDES permit experiences of Virginia and Maryland. What are the species they use and what are their appraisals?

Maryland NPDES species:

	Acute	Chronic
Freshwater:	*Daphnia*	*Ceriodaphnia*
	Fathead minnow	Fathead minnow
Brackish:	Sheepshead	Sheepshead
	Silversides	
	Grass shrimp	
	Mysid shrimp	Mysid shrimp

Neomysis has also been used.

Virginia NPDES species:

	Acute	Chronic
Freshwater:	*Daphnia pulex*	*Ceriodaphnia*
	Fathead minnow	Fathead minnow
Brackish:	Sheepshead	Sheepshead
	Mysid shrimp	Mysid shrimp
		Champia

Have *Fundulus* and grass shrimp also been used?

Other considerations. We should also use ecological indicator (keystone) species, as well as species amenable to laboratory testing. There is some overlap: some of these amenable species are also ecologically significant species (for example, grass shrimp, *Neomysis, Mysidopsis*). Some good test species are only available on a seasonal basis, for example striped bass larvae, which is an important species and sensitive stage. In using lab species, we must maintain "ferality" to assure that we're not testing genetically different organisms from feral species. We should also use some species that are common to both lab and effluent testing. Data comparing the sensitivity of the silversides/sheepshead minnow to some of the indigenous species of the Bay are incomplete, but mysids appear to be preferable for chronic testing. For an acute test for finfish, Menidia and striped bass should be considered.

One could argue for conducting chemical testing when no ambient toxicity is shown, but to keep living species as good indicators of toxicity.

Question: What comprises the list of chemicals present in the Bay, and do these species reflect levels of these chemicals?

Response: The EPA's National Water Quality Database would be the source for that information.

Purposes intended for these tests. The suite of organisms will be vastly different depending on whether the study has regulatory endpoints. Our test methodologies will be designed to meet the goals of the Toxics Reduction Strategy. In our considerations, we will combine elements of both more-standardized tests in the regulatory sense and broader-based tests and methodologies. We will address the general water quality considerations of Chesapeake Bay, recognizing that specific sites indicating toxic point source problems will be addressed directly by other regulatory testing tools (e.g. NPDES).

Point: Acute lethality tests for Bay species are not sensitive indicators of ambient Bay toxicity and should not be used routinely as such. Chronic tests or partial chronic tests with sublethal indicators are preferred. In areas where you expect high toxicity, you may want to use acute lethality testing as an initial screen.

Agreement was made to establish three categories of tests:

1. Established regulatory methods

2. Methods for less commonly used indigenous species

3. Methods for indigenous species, still within research mode.

We also have three environments to consider:

1. Freshwater (0 ppt salinity)

2. Estuarine (> 0 to 20 ppt)

3. Marine (> 20 ppt)

We need to have (1) benchmark species and (2) controls/references of water with matched salinity, both of which can run through testing procedures.

Choice of control sites. The lower Bay on Maryland's Eastern Shore is cleaner (based on demogra-

phy); thus it is a candidate for control sites/reference area. Discussion on use of reconstituted waters included the caution that you
may add back higher levels of metals from artificial sea salts than the levels you would be testing for. Further options were suggested for sources of control water.

- Use water from Punch Island Creek, a relatively unspoiled refuge on lower Eastern Shore, with high oyster seed population.
- Filter Bay water through a well site to be established on a beach.
- Consider multiple sites of varying salinities, especially critical habitat requirement areas.
- Filter ocean water. Caution: diluting natural seawater with distilled water gives ionic variance that is not representative of ambient low-salinity samples.
- Control for each site or range of area? In this case there must be general guidelines.

Question: Where will the tests be conducted?

Response: Tests will be done on a multi-institutional basis and in mobile labs.

Slide: Bay water flow observations made at one point showed six circulation patterns, with varying percentages of frequency of occurrence. Water samples over ten days yield an average of these patterns, which is the dominant circulation. This variability should be considered during ambient water toxicity testing.

Response: We try to get at this integrative function for water patterns by looking at the animals -- that's why we do *in situ* testing.

Point: The longevity of a monitoring program is inversely related to the individual cost per sample; high costs ensure short-lived programs.

Response: In initial studies it may be necessary to oversample so that your long-term program does not overlook significant factors. It is important to make the best use of this pilot study.

Costs of composite samples may be worrisome for ambient testing procedures. There are basic questions on how to collect these samples. Two options are seen: grab sampling at various depths, and composite sampling. For both methods, both cost and design must be considered. EPA's Technical Support Document outlines the pro's and con's of these sampling methods.

Stationary vs. mobile laboratories. It was agreed that there are inherent differences in the use of species in stationary vs. mobile laboratories. Mobile toxicity labs, on-site, have two advantages: (1) avoidance of long-term sampling storage and (2) immediate testing. All lab species identified previously are suitable for mobile labs except: (1) some fish, depending on age and species (and vulnerability during transport) and (2) spawning stock.

***In situ* studies.** Definition: *In situ* refers to an organism immersed directly in the ambient water enclosed in a screened or other type of chamber. Problems with research-oriented species may be magnified by in situ complications. Expected problems include general problems with egg and larval stages (most cannot be used) and problems with *Xenopsis* adult stage (air-breathing) animals.

Appropriate freshwater species include :

- Bluegill
- Striped bass
- Catfish
- Fathead minnows

- ?White perch

Appropriate estuarine species include:

- Sheepshead
- *Menidia*
- Mysid shrimp

In observing adult stages *in situ* we won't necessarily observe chronic toxicity; therefore we must be sure that the effects observed are from contaminants and not from the physical setting. Three factors can cause problems: salinity, temperature, and food supply.

Suborganismal testing has potential for use in ambient toxicity testing. Some tests may function as early warnings, some as indicators of a specific stressor, and some as indicators of classes of compounds inducing reactions. However, not all methods are yet well-established. These methods must be properly validated and documented before they are applied to field conditions.

Types of suborganismal tests:

- Immunological suppression
- Cytochrome P450 (a fairly good data base is associated with this)
- Metallothioneins (these are good indicators of metal regulation, especially Cu and Zn)
- RNA/DNA ratios in larval fish in feeding experiments

  Stress proteins (these offer a universal response to stress, regardless of species; often single or a few organisms are sufficient for testing).

Question: Can we use biomarkers in trend analysis?

Response: If we do so, we should use a suite of responses rather than a single one.

Question: Is a reference toxicant test applicable to biomarkers; are they used as such?

Response: We need to establish comparable databases for all biomarkers. Future goals will include gene studies on the source of protein synthesis and the initiation of transcription.

Question: Should we use biomarker methods in the pilot study when they are still in the research phase?

Response: We shouldn't discount them, because they can indicate whether the system is getting better or worse. They can act as a forensic approach to toxics analysis. If we include them in the pilot study, then both the specific and general information obtained will be available for linkage to specific toxics.

Costs. We need to further address question #3 dealing with the costs. Maryland and Virginia will provide us with the cost data associated with their programs for regulatory methods (NPDES). No definite estimates are possible with in situ testing; they are very site-specific. If we are given the parameters, then we can estimate.

Clarifications of category distinctions:

- Proven regulatory methods
- Chesapeake Bay-specific established methods
- Chesapeake Bay research methods

Water quality and contaminants data. We must include the general water quality considerations, i.e., DO, salinity, temperature, etc. The in situ testing allows for continuous measurements over long periods of time. Testing considerations will be driven by/restricted by the relative costs. Accurate testing will

require background data review to narrow down the number of toxicants to consider.

Comment: Toxicity should drive the types of analyses done. We should be careful not to prejudge the presence/absence of toxicants; we should allow the testing to prove this.

ICP (inductively coupled plasma emission spectroscopy) analysis could be coupled with CBP (Chesapeake Bay Program) water quality analysis approaches, assuming that detection limits are appropriate for ambient analysis.

We should emphasize the importance of the overall coordination of investigators to avoid duplication of testing efforts. We recommend panel review of sample collection, perhaps through an RFP process:

- conducting sediment contaminant analyses with water column
- contaminant analyses
- using composite sampling and grab sampling.

Comment on the recommendation in the Statement of Purpose (p. 6), "Samples should be preserved and analyzed only when biological test results are positive for toxicity:" If levels of toxicants are found in the system and organisms still do well, this is an important aspect of the results and should be included. The contaminants measured that show no toxicity are important.

Question: Will a decision on the pilot study consider the science equally with the socio-political considerations?

Response: I don't think we're locked into the proposed study areas. Steve Jordan mentioned yesterday that the area suggested for the pilot study was basically to promote discussion.

Water sampling

We need:

- Consistency in the *in situ* testing procedure to be able to compare the responses of organisms.
- Lab analyses of grab samples are standard methods, but we will emphasize the use of composite samplings.

Funding considerations

Point: We need to come up with realistic methodologies based on budget restrictions.

Response: Our primary concern is to determine the best science to apply to the problem. Budgetary considerations come after that. We should not focus on the amount of money available but rather the proper methodologies to be used.
Response: The number of sites has not been decided and will depend on the funding available. Continual efforts are being made to increase the monies available. The letter to EPA Administrator William Reilly from 25 Bay-wide researchers made the point that the presently authorized monies are not sufficient to adequately answer the toxics questions at hand. We may not know until September what money will be available in October.

Suggestions:

Match the pilot study stations to already-established Bay-wide monitoring program stations.
Exploit *in situ* techniques (for instance, simple oyster "trees" some years ago yielded a lot of information about vertical variations in both concentrations and uptake of metals).

Incorporate histopathology, which can indicate effects on health of Bay organisms. Sample

indigenous fish and invertebrates within the pollution gradient of the pilot study.

Presentation of risk assessment group's conclusions was made by Ian Hartwell (see Risk Assessment Summary).

Discussion

Comment: It seems that the community was emphasized in the risk assessment discussion rather than the organism/species emphasis that was evident in the water column discussion.

Response: Yes, because tests done on the organismal/species level often are not done in a manner that allows the data to be used for risk assessment. We must be able to extrapolate this data to estimate the integrity of the community.

Question: Did you address in your group the operational requirements of a pilot study, i.e., personnel, expertise, and time?

Answer: We tended to focus more on a long-range program. We decided it would take a number of years and work-hours to establish program databases. Further information to consider would include modeling links, which may require specialized people.

Question: Do you have an existing model available?

Answer: With the present pilot study restraints, there are no good estimates of chemical exposure. If we had exposure data or could generate data (which would require a substantial amount of money), or if we could collate for Chesapeake Bay data from other data bases, then we could apply these to the estimations of chemical exposures. We can't do risk assessment without exposure data.

Question: What types of chemical exposure data do you need?

Answer: We'd be looking for cause/effect-type studies, including subcellular, suborganismal, and whole organism information. It would be valuable to be able to target areas of toxic concern (hot spots) and be able to apply this data to a long-term approach.

Question: What are your abilities in predicting a risk associated with a defined hot spot in the Bay system?

Answer: If we can measure the level of harm in a species or group of species, then through cause and effect modeling or extrapolation modeling we can create a probability function that we can apply to the community via extrapolation.

Comment: A number of existing programs already have data from monitoring activities, and we should draw from these for our considerations here.

Comment: EPA has asked CRC to collect all the historical data available on Bay toxics, and the results of this collection will be made available next year.

Notes on *in situ* selection

Livingston et al. (1986) used benthic "microcosms" (colonization plates, sediment traps) to transfer communities between clean and contaminated areas. These experiments could be useful in *in situ* tests for toxicity. Such experiments imply relatively long exposures (probably 14-30 days).

Pratt et al. have transplanted artificial substrates in streams, moving communities from reference sites to "impacted" sites, and have measured loss of species, decreased biomass, changes in nutrient content, and nutrient transporting enzymes. This work is in review (in part) with ASTM Aq. Tox. 13th Symposium.

Questions Related to Sediment Toxicity Assays

Considering that the primary management need of the Chesapeake Bay Program related to this topic area is to identify sediments that are contaminated to the point where living resources are impaired (either by direct contact, ingestion, or fluxes of toxicants into the water column), the following questions should be addressed:

1. What routes of exposure should be assessed? (e.g. ingestion by deposit-feeding fauna; direct contact by epibenthic fauna; ingestion of suspended solids by filter feeders; direct/indirect contact by fish; bioaccumulation and food chain accumulation, etc.)

2. What types of assays are currently available to assess the effects of these routes of exposure?

3. What is the relative feasibility of these assays in terms of costs and technical ease (i.e. time, training, and specialized facilities requirements) in a large scale monitoring program?

4. What species should be selected for the assays? (Consideration should be given to practicality, cost, and sensitivity, as well as importance to and representativeness of living resources in the Bay.)

5. What biological endpoints should be selected? (They should be relatively rapid, sensitive, interpretable in terms of risk to living resources, and, hopefully, display continuity with "whole organism" and "suborganismal" assays being discussed by other work groups for assessing ambient water and effluents.)

6. How can sub-organismal measures be incorporated into traditional acute or chronic toxicity tests? How can results be interpreted in terms of impact on living resources and their habitats?

7. What sort of experimental design(s) should be adopted in a "full scale" assessment of the Bay? (i.e. sites selected randomly, uniformly, or on a stratified random basis; the number and definition of "reference" or "control" sites; areas that should be targeted for special effort; the role of a tiered approach, etc.)
8. How can the results of the assays be integrated with other monitoring data to "feed" into a Chesapeake Bay Risk Assessment Strategy?

a. What sort of chemical monitoring would be needed to support this effort?

b. What sort of biological monitoring of in situ living resources would be needed to support this effort?

c. What other information would be required?

Workgroup Sessions on Sediment Toxicity Assays

Convener: Ray Alden
Chair: Richard Peddicord

There was a general discussion about how to design a strategy of sediment toxicity testing in the Potomac Pilot study, the feasibility of such a study given the budgetary constraints, and whether the Potomac was a suitable place to develop testing strategies applicable to the whole Chesapeake Bay.

Question: How do we use, set up, and apply these tests to the Bay? (Question 7 was chosen as the beginning point in discussion.)

The pilot program can be used to field-test both standard and modified-standard techniques and to develop a basis for recommending specific, more chronic, estuarine-based techniques. Cost factors must be assessed at the same time.

Sediment quality criteria. It is agreed that sediment quality criteria (SQC) are needed, but consensus is lacking on how they should be used and implemented.

Database development. Development of SQC will require a large database. A database should be developed which will also support future application of SQC and/or other sediment quality evaluation techniques. Parameters for such a database are presently being collected and observed, but coordination is needed in the gathering of this information. A database can be developed that will aid in developing SQC. Some work in the Elizabeth River is being done to relate toxicity tests to SQC.

Consensus: We need to collect supporting data for SQC when doing toxicity tests and chemical analysis, to the extent that we know what these data are. W e need to compile not only the relevant sediment chemistry, benthic data, and toxicity data, but also data on grain size, outfall locations, sources of contamination, etc., and use this information to guide the Program.

Pilot study siting

Question: How did the Potomac River get chosen for the pilot study?

Response: The research was already done on it, it spanned political jurisdictions, and it was an integrated estuarine system (encompassing tidal freshwater through mesohaline water).

The question arises whether sediment toxicity is causing problems for living resources. If not, then what is the use of studying sediment toxicity? Living resources are the focus of public attention. For instance, the striped bass is still in trouble in the Potomac, but there is public pressure to open the fishing rights again, because the fish larval index has improved slightly.

Question: If the Potomac is relatively clean then why look for sediment toxicity there? Not much sediment data exists. It would make sense, if the Potomac is studied, to include a comparison of the Elizabeth River to the Potomac: worst case compared to relatively clean. This would also provide verification that the methods used in the Potomac do work in a site where we already know there is toxicity.

Consensus: A positive control should be established, perhaps outside the Potomac system, to demonstrate that the tests being run do work.

Comment: The budget for the entire pilot program is $250,000, for all aspects of the study.

Question: What is the relation of sediment toxicity to the striped bass fishery?

Response: Answering this question probably involves looking at other resources, i.e., benthic species.

Question: Do we know what tests and present body of knowledge exist for the quality of Potomac sediments?

Response: The D.C. Department of Environmental Control has recently conducted benthic community structure analyses for several sites in the upper tidal Potomac and Anacostia Rivers. These studies showed most of the Potomac sites to have fair health indices, with some in the moderately good range. All sites in the Anacostia reflected poor indices. A continuing study of Anacostia sediment toxicity has found considerable sediment toxicity, particularly at the mouth of the Anacostia. The recently improved health of the upper Potomac has been partly attributed to the water filtering capacity of the clam, Corbicula, and the return of SAV, which has encouraged the return of several fish species.

The Great Lakes Program used a suite of tests to determine areas of concern as part of the ARCS

program, and then employed bioassays to find sediments which were better or worse in quality. These tests included:

- sediment grain size fractions
- organic carbon
- solvent extractables
- organically-bound chlorine, bromine, and iodine
- inductively coupled plasma (ICP) analysis of selected metals
- Microtox bacterial luminescence assays (perhaps not appropriate to the Bay given the narrow salinity range restrictions).

Perhaps we should be looking for new and innovative measures that will give us more complete information. Many of the standard tests applied to sediments have historically been adapted from old water quality tests. There is a real need to develop new tests aimed specifically at sediment quality testing needs.

Identification of sites in the Potomac. We need to use existing information to identify areas where possible sediment toxic effects might be. We need to do toxicity testing on both the very dirty and the clean sites, as well as the gray areas in between. We need both positive and negative reference sites so that we can establish the outermost ranges of both natural survival and the anticipated biological endpoints. Establishing such a gradient, encompassing the range of toxicity, would also allow us to establish or measure the relative sensitivity of the toxicity tests.

Comment: The charge is to determine the effects of toxics on living resources of the Bay.

Comment: We should start with benthic community structures to identify what areas are of concern. Since this is a demonstration project, we will have to show responses across a contaminant gradient.

Question: What are we going to set as a biological impact level? What level of reduction in survival is statistically significant?

If the sediment bioassays (acute tests) tell us that the benthic area is disturbed, we still can't make large leaps from sediment bioassays to living resource risk assessment. But we can make the connection from sediment tests to benthic community structure effects.

Variability. Ecologists admit that there is large variability in benthic community data. Many factors can cause a disturbance; it is hard to attribute a change to one or two factors. The best approach is to look at all three components, i.e. sediment chemistry, toxicity, and benthic community, to get a handle on sediment quality.

It is important to identify what we will consider as a statistically significant impact. The protocols should specify that design and sampling schemes be set to get results that show xx% response that signifies a significant difference.

There are two types of variability: (1) natural variability in sediment quality that can be partially overcome by compositing sediment samples from a site, and (2) variability due to the precision level of the test method and variability of species. Amphipod tests show that differences of 15% are usually significant. We need a more qualitative examination of benthic community structure before we can assess the importance of the variability that is observed.

Although funding constraints may limit how much sampling can be done, the problem with using one sample per site is that you don't learn anything about

within-site variability. We need to determine the variability in endpoints and ecological conditions; this would require a suite of tests in one area.

We should use a fairly quick, cheap screening tool, then use the toxicity tests. The reproducibility and power of the test should be kept in mind when interpreting results. Data bases are available for making estimates on the level of replication that will give differences.

Question: Should within-site variability be our focus?

The pilot parameters will determine the site, species, and variability. The power of the test must be recorded and verified during study.

Once we have done sampling then we can get a handle on the variability. We should use species that have demonstrated sensitivity; we should test whole sediments and then determine which tests can be applied to the Bay.

Question 2: "What types of assays are currently available to assess the effects of these routes of exposure?" Basic recommendations are to use whole sediment methods and to test species that have some existing database.

Possible tests.

Standard acute

10-day amphipod test for screening (there are amphipods, *Lepidactylus* and *Leptocheirus*, for example, that should be used and developed).

The basic screening tests that have been fairly cost effective for the Great Lakes.

Suggestions:

- Use biological tests (benthic community structure) first to screen, then chemistry testing and sediment bioassays.
- Use a suite of test species and methods.
- Use infaunal species rather than epibenthic ones.
- Demonstrate the utility of an amphipod test for the Bay using existing standard methods and species indigenous to the Chesapeake Bay.
- Develop and field-validate tests for chronic and population effects of toxic sediments using Chesapeake Bay indigenous species.

The primary goal of the Chesapeake Bay Agreement is to protect living resources. However, our knowledge of the impacts of toxics in the Bay is weak. This workshop should provide the guidelines for toxics assessment. The drafters of the Agreement did not know a great deal about sediment toxicity.

A question we haven't addressed is freshwater toxicity testing. The relative sensitivity of species across salinity gradients is important. We should include this in our recommendations.

Selection of species. Discussion on standard tests and tests in development that are appropriate for the various salinity levels of the Chesapeake Bay is summarized in a table (see Final Plenary).

A test showing promise, but still in development, is one using Sago pondweed (*Potomageton pectinatus*). It was at one time the primary food for many migratory birds, but after being historically present it has disappeared. Using tissue culture techniques, it can be produced year-round. The test takes about 4-6 weeks, as it measures mortality over time; several endpoints can be used. It is of interest because it represents a problem group (SAV), and because it represents the primary producer level.

Standard protocols must be set forth for sediment toxicity testing for the Chesapeake Bay and should be required for use by all pilot programs and final program investigators.

Recommendations. Before testing begins we must have a quality assurance/quality control plan and develop standard operating procedures for the Chesapeake Bay. These should address the following:

- sediment collection and storage
- extraction procedures
- test procedures.

Bioaccumulation

Question: How should bioaccumulation be considered and evaluated?

If we use fish monitoring, we should try to select a species that has been shown to accumulate through a sediment source. However, routine monitoring of bioaccumulation may not be pertinent to the charge of the program as a threat to living resources. There should be a demonstrated problem due to bioaccumulation in an area before we say it's necessary to study it. Widespread studies would not be cost-effective. Both Maryland and Virginia will continue to do bioaccumulation/tissue residue monitoring focused on edible fish and shellfish since it's such a huge economic issue. If these programs find a problem, then we will have a justification for looking further into it. We can examine the living resources and try to identify whether the toxics are coming from the sediments or the water.

We should define an area of sediment sampling and limit it to the 10-year flood plain. Some tidal plains and marshes have some of the worst problems with toxic sediment.

Sub-aquatic, water-saturated sediment must be considered separately from soils.

Protocols for sediment depth sample. Based on the specific hypotheses/questions being stated/asked, the depth of sediment collected for toxicity testing must be clearly defined before implementation of the pilot program. Factors to be considered include focus on recently deposited sediments, average depth for benthic infauna (10-25 cm), depth of dredging (dependent on site), and a historical review of sediment toxicity (core sectioned by depth according to local depositional patterns).

The population risk assessment group has tried to come up with a ranking of sites, with sediment toxicity as one factor.

Question 6 -- How can suborganismal measures be incorporated into sediment toxicity work?. The workshop participants working in this field will incorporate some material from the SETAC meeting into this report.

We could use pore water tests in conjunction with cross-media testing. We should give priority to cross-media methods. Sub-organismal methods are best tied in with chronic tests. Metabolic enzymes will help in the "fingerprinting" of sources of toxicants. Some genetic work has been done in Michigan with mollusks, and although problems remain in extrapolating suborganismal effects all the way to population risk assessment, the work has potential. It could be useful to poll the medical community for ideas on extrapolation from sub-organismal to environmental risk assessment.

Sublethal effects may be good tools to assess causative factors of toxicants, although there are problems of covariance. Sediment mixtures of contaminants that are separated do not always behave the same as the original mixtures, due to additive effects. The attribution of toxicity to a specific toxicant by means of these tests may prove easier in the water column than in the sediments.

Sublethal effects can also be used to track subtle changes in organisms. A sublethal effect is an early warning signal that can be connected to growth and reproduction effects.

There are two classes of responses: (1) a stress-protein response to general stress and (2) other responses that can be used for specifically identifying a responsible contaminant. These two classes of response may be used in a tiered system. An assay at tier 1, e.g., a stress-protein assay, will determine an effect, and an assay at tier 2, e.g., metallothionein or cytochrome P450, will identify what is causing it. We are reaching the point where the answers to specific toxicity can be teased out, but it is somewhat tricky.

The economics of this kind of tiered approach are good, as these assays are much cheaper than routine sediment chemical analysis. Some labs can run these tests on a routine basis.

There is need for an entire suite of tests, not just the standard amphipod assays. However, polychaetes seem to be less sensitive, and whole-sediment exposure tests are better developed for amphipods. We are hampered by the fact that appropriate tests for sediment in other phyla haven't been thoroughly developed and tested, and we are working on a short time budget.

For risk assessment, endpoints of mortality, fecundity, and growth and bioenergetic measures are most helpful. The relative sensitivities of species and endpoints are important for statistically generated models. From a managerial standpoint, looking at species representative of the Bay is important. A manager would want to relate tests to *Callinectes, Crassostrea,* and important fish species.

The exposure of animals is difficult to assess in species you aren't familiar with. Also, the animals chosen have to be maintained in labs. This necessity will restrict the species selected to those that are robust enough for this handling, yet also meet all the other criteria of sensitivities needed for sediment testing. Since the relative sensitivities of benthic invertebrates aren't known, then toxicity tests should be coupled with benthic community analysis.

There is need for a gradient of contamination in the pilot testing, and many have doubts whether the Potomac offers this gradient. The problem of finding a true uncontaminated or no-effects reference site(s) will be difficult but also critical.

A standard reference sediment may be needed so that all toxicity tests are run with the same reference. The selection of reference sites will depend on the objectives of the Program. If the objectives are to assess relative contamination within the Bay, then this information will evolve from data collected at all sites tested. Strict hypothesis testing would require more stringent reference definition.

Recommendations

Suborganismal endpoints should be developed. However, they are not ready for immediate use in this Program; first they must be directly related to whole-organism effects.

The Program needs specific, focused objectives and testable hypotheses before the question of reference sediment and sites can be adequately addressed.

Appendix B

Workshop Participants

Methodologies for Whole Organism Toxicity Testing

Plenary Speakers:

Mr. Steve Schimmel
Environmental Research Laboratory
U. S. Environmental Protection Agency

Dr. Jeffrey Black
Graduate Center for Toxicology
University of Kentucky

Workgroup Chair:

Mr. Steve Schimmel
Environmental Research Laboratory
U. S. Environmental Protection Agency

Convener:

Mr. Lenwood Hall
Applied Physics Laboratory
The Johns Hopkins University

Workgroup Participants:

Dr. Brian Bradley
University of Maryland
Dept. of Biological Science

Dr. Arthur Butt
Virginia Water Control Board

Dr. John Cooney
Battelle Memorial Institute

Dr. David W. Engel
NOAA/NMFS
SE Fisheries Center
Beaufort Lab

Dr. Jay Gooch
Chesapeake Biological Laboratory
University of Maryland

Dr. David Gruber
Biological Monitoring Inc.

Mr. George Kennedy
Hampton Roads Sanitation District

Dr. Arthur Ott
Susquehanna River Basin Commission

Mr. William Pfeifle
Virginia Water Control Board

Dr. John Pritchard
Laboratory of Pharmacology-NIEHS

Dr. G. Roesijadi
University of Maryland

Chesapeake Biological Laboratory

Mr. Keith Sappington
Maryland Dept. of the Environment

Mr. John Veil
Maryland Dept. of the Environment

Dr. Dave Wright
Chesapeake Biological Laboratory
University of Maryland

Recorder:

Ms. Robin Laird
Chesapeake Bay Liaison Office

Sediment Toxicity Assays

Plenary Speakers:

Dr. K. John Scott
Environmental Research Laboratory
U. S. Environmental Protection Agency

Dr. Chris Zarba
U.S. Environmental Protection Agency

Workgroup Chair:

Dr. Richard Peddicord
E A Engineering Science and Technology

Convener:

Dr. Ray Alden
Applied Marine Research Laboratory
Old Dominion University

Workgroup Participants:

Dr. H. Suzanne Bolton
National Oceanic and Atmospheric Administration
Washington, DC

Ms. Sherri Clark
Virginia Water Control Board

Dr. Jim Flemming
US Fish & Wildlife Service
North Carolina State University

Dr. Michael J. Mac
Great Lakes Fishery Lab
U. S. Fish & Wildlife Service

Mr. Eli Reinharz
Maryland Department of the Environment

Ms. Debra Trent
Virginia Water Control Board

Recorder:

Ms. Kim Warner
Chesapeake Biological Laboratory
University of Maryland

Population Risk Assessments Based on Toxicity Testing

Plenary Speakers:

Mr. Donald Rodier
U. S. EPA, Office of Toxic Substances

Dr. Glenn Suter II
Oak Ridge National Laboratory

Workgroup Chair:
and Convener:

Dr. Ian Hartwell
Horn Point Environmental Laboratories
University of Maryland

Workgroup Participants:

Mr. Charley Banks
Virginia Water Control Board

Dr. Katherine Farrell
Environmental Science and Health
Maryland Department of the Environment

Dr. Yacov Haimes
Department of System Engineering
University of Virginia

Dr. Kenneth Jenkins
CBR, Inc.

Ms. Gail MacKiernan
Sea Grant Program
University of Maryland

Dr. Robert Otto
Otto & Associates

Dr. Kenneth Perez
EPA, ERL, Narragansett

Mr. Mark Richards
Virginia Water Control Board

Dr. Robert Ulanowicz
Chesapeake Biological Laboratory
University of Maryland

Recorder:

Ms. Jackie Savitz
Chesapeake Biological Laboratory
University of Maryland

Methodologies for Sub-organismal Toxicity Testing

Plenary Speakers:

Dr. G. Roesijadi
Chesapeake Biological Laboratory
University of Maryland

Dr. Jay Gooch
Chesapeake Biological Laboratory
University of Maryland

Conveners:

Dr. Jay Gooch
Chesapeake Biological Laboratory
University of Maryland

Dr. Peter Van Veld
Virginia Institute of Marine Science

Additional Workgroup Participants
(Day 1 Plenary and Workgroup "floaters")

Mr. Dan Audet
Chesapeake Bay Estuary Program
U. S. F. W. S.

Mr. Richard Batiuk
U. S. Environmental Protection Agency
Chesapeake Bay Liaison Office

Mr. Mark Bundy
Maryland Dept. of Natural Resources

Dr. Dennis Burton
The Johns Hopkins University

Dr. William Busey
Experimental Pathology Laboratories

Ms. Liz Conner
University of Maryland

Dr. Eugene Cronin
Private Consultant

Dr. Rex D'Agostino
University Micro Reference Lab

Dr. Tom Dillon
Waterways Experiment Station
U. S. Army Corps of Engineers

Ms. Phyllis Frere
PEPCO-Hallowing Point Lab

Ms. Elaine Friebele
Interstate Comm. on the Potomac River Basin

Ms. Mary Jo Garreis
Maryland Dept. of the Environment

Ms. Bess Gillelan
Estuarine Programs Office

Ms. Eileen Hamilton
Chesapeake Biological Laboratory
University of Maryland

Mr. George Harmon
Maryland Dept. of the Environment

Mr. Carlton Haywood
Interstate Comm. on the Potomac River Basin

Ms. Paula F. P. Henry
USFWS-Patuxent Wildlife Research Center

Mr. Lance Himmelberger
Penna. Dept. of Environmental Resources

Mr. Jerrald Hollowell
Susquehanna River Basin Commission

Mr. M. Paul Jackson
Martel Laboratory Services

Dr. Margaret O. James
Department of Medicinal Chemistry
University of Florida

Ms. Betsy Johnson
Water Hygiene Branch
District of Columbia Government

Mr. David Jordahl
Maryland Dept. of Natural Resources

Dr. Stephen Jordan
Maryland Dept. of Natural Resources

Dr. Andy Kane
School of Medicine
University of Maryland

Mr. Stuart Lehman
Chesapeake Bay Foundation

Ms. Laura Lower
Virginia Council on the Environment

Ms. Patmarie Maher
Estuarine Studies Office

Dr. Joseph Mihursky
Chesapeake Research Consortium
University of Maryland

Dr. Jack Plimmer
USDA, Agricultural Research Service

Mr. Alan E. Pollock
Virginia Water Control Board

Dr. Richard Pratt
School of Forest Resources
Pennsylvania State University

Mr. David Pyoas
Maryland Dept. of Natural Resources
Dr. Andrew Robertson, Chief
Ocean Assessments Division
NOAA/NOS, N/OMA3

Mr. John Slowikowski
Industrial Discharge System
Maryland Dept. of the Environment

Dr. Roland C. Steiner
Interstate Comm. on the Potomac River Basin

Ms. Cynthia Stenger
Maryland Dept. of Natural Resources

Mr. James T. Ulanoski
Penna. Dept. of Environmental Resources

Dr. Wolfgang K. Vogelbein
Virginia Institute of Marine Science

Dr. Clarence Wade
University of the District of Columbia

Dr. Lloyd Wolfinbarger, Jr.
Center for Biotechnology
Old Dominion University

Dr. Chris Zarba
U. S. Environmental Protection Agency

Workshop Staff
(All staff were employed by the Chesapeake Research Consortium.)

Ms. Karen McDonald
CRC Project Manager

Ms. Dana Flanders

Ms. Pam Owens

Ms. Cindy Corlett

Ms. Elizabeth Krome

Ms. Deb Young

Ms. Elizabeth Egeli

Appendix C

Bioassay Capabilities Survey Results

The information for this appendix did not come directly from the workshop, but was collected by workshop staff and reflects the recommendations made by the workshop steering committee. An introduction follows which explains the purpose of this addition and the process by which it was developed. A separate page of acknowledgements is included as well.

ACKNOWLEDGEMENTS

Thanks are expressed to Cindy Corlett, Pam Owens, and Jackie Savitz for their coordinated efforts to develop the questionnaire, contact labs, enter response data, develop a compilation format, and edit the final product. Gratitude is also extended to the representatives from the labs listed below who gave their time to complete the questionnaire.

Participating Laboratories

Academy of Natural Sciences

Applied Marine Research Laboratory

Aqua Survey, Inc.

Biological Monitoring, Inc.

Bionetics Corporation, Analytical Laboratories Division

Center for Environmental Studies

Chesapeake Biological Laboratory

Coastal Bioanalysts, Inc.

Commonwealth Laboratory, Inc.

EA Engineering, Science, and Technology, Inc.

Environmental Laboratories, Inc.

Environmental Resources Management, Inc.

Environmental Systems Service, Ltd.

Experimental Pathology Laboratories, Inc.

Free-Col Laboratories, Inc.

Hampton Roads Sanitation District

Horn Point Environmental Laboratory

James R. Reed and Associates

Johns Hopkins University, Applied Physics Lab

Malcolm Pirnie, Inc.

Olver Incorporated

Patuxent Wildlife Research Center

Riverside Laboratories

Technical Testing Laboratories

University of Maryland, Agricultural Experiment Station

University of Maryland at Baltimore, Aquatic Toxicology Facility

University of Maryland, Baltimore County

Versar, Inc. ESM Operations

Virginia Institute of Marine Science

INTRODUCTION

About the survey

This appendix is the product of a survey conducted by the Chesapeake Research Consortium, Inc. (CRC) September through December of 1989. This survey was conducted to determine the capabilities available for conducting bioassays within the Chesapeake Bay watershed. A questionnaire was developed and sent to all laboratories in the region who were identified as possible candidates. Fifty-one laboratories in Virginia, Maryland and Pennsylvania were sent questionnaires and explanatory cover letters. Of these, 29 laboratories participated in the survey: the responses from their completed survey forms comprise this appendix.

The remaining laboratories did not respond for a variety of reasons. Some questionnaires were returned to CRC by the post office. Some lab managers reported that they did not conduct applicable tests. Others simply did not respond. A listing of these laboratories and their response status is available from the Chesapeake Research Consortium upon request.

About this compilation

This booklet is divided into two sections. The first is a series of one- to two- page entries which are arranged alphabetically by lab and begin on page 47. These entries contain general information about the laboratories such as lab address, a contact person, types of bioassays conducted, field sampling capabilities, accessible analytical chemistry equipment, computers and software commonly used, staff available, and data on respective quality assurance/quality control programs. The format of these pages follows that of the original questionnaire closely. Likewise, care has been taken to enter the labs' responses as closely as possible, notwithstanding necessary editorial changes.

The second section is arranged by type of bioassay, giving specific information about the bioassays conducted at each lab; it begins on page 205. There is a table for each of the following types of bioassays: acute fish, acute invertebrate, bacterial, biochemical ("biomarkers"), chronic fish, chronic invertebrate, plant (algal), and sediment. Entries within each table are listed alphabetically by lab and include information on species, test conditions, organism's life stage, test length, salinity, lab's holding capacity, test turnaround time, and the number of bioassays each lab can run simultaneously.

Future assessment

It is our hope that these results will be utilized both as a catalog of the services available for contracting and as a data compilation from which planners can determine areas to target for future development.

Academy of Natural Sciences

James Sanders
Benedict Estuarine Research Laboratory
Benedict, Maryland 20612
301-274-3134
FAX 215-299-1199

Testing site: Benedict Estuarine Research Lab.
Benedict, Maryland 20612
Hours: 8:00 AM - 4:00 PM

Bioassays Conducted

Types of bioassays: The Academy of Natural Sciences does not do conventional bioassays. However, much of their research is devoted to assessing the impact of low levels of toxics on natural estuarine communities. Many of these studies result in techniques useable for toxicity assessment. For example, they maintain a microcosm facility that enables them to investigate toxic impact to phytoplankton communities. They also routinely maintain 30 or so algal species in culture, which could be utilized for assays. (See tables for description of exact test capabilities.)

Future plans: The Academy will continue with the same areas of research.

Field sampling capabilities: sampling gear for water column, organisms and sediments; variety of vessels, 20-45 feet

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty:
- trace elements; particularly organ metalloids
- organics; analyzed by sister lab in Philadelphia

Major equipment available:

electron microscopy:	none reported
spectrophotometry:	2 AA's for elements in Benedict
	MS
	GC/LC MS in Philadelphia
chromatography:	GC in Benedict
	GC, LC's in Philadelphia

Additional Resources

Computerized information retrieval services available: DIALOG

Computers and software available:
- usual mix of personal computers and software
- VAX 11/730 running VMS and SAS

Staff available: 6 Ph.D.- level senior scientists
20 B.S./M.S. support staff

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

(Only in compliance at Philadelphia Lab.)

Follow study protocols? N/A Have a complete set of Standard Operating Procedures? N/A

Archive facility for the data generated? N/A

Computer-generated data? N/A Are these computer systems validated? N/A

Applied Marine Research Laboratory

Old Dominion University
Dr. Raymond W. Alden, III
4401 Powhatan Avenue
Norfolk, Virginia 23529-0456
804-683-4195
FAX 804-683-5293

Testing site: AMRL
Research East Building
Center for Biotechnology
Dept. of Biological Sciences
Mills Godwin Bldg.
Old Dominion University
Norfolk, Virginia 23529

Hours: 24 hrs./day

Bioassays Conducted

Types of bioassays: The Applied Marine Research Lab (AMRL) conducts acute and chronic bioassays on fish and invertebrates as well as a suite of sediment-organism bioassays. (See tables for descriptions of exact test capabilities.)

Other bioassay work: Toxicological and biotechnological assays of various sorts are now being developed at ODU. Cell culture and microbial cultures are being developed for extensive screening activities; histopathological, behavioral, genetic and teratogenic assessment are being developed for testing subtle chronic effects of toxicants on aquatic organisms.

Future plans: AMRL and the Center for Biotechnology plan to continue development of a toxicology emphasis, developing sensitive, cost-effective toxicity tests capable of high capacity sample through-put. Flow-through and mobile/on-site capabilities are also being developed.

Field sampling capabilities:
- full range of collection protocols and gear for obtaining organisms, water, sediment, and wastewater samples
- fleet of research vessels from small (10-20 ft.) and mid-size (20-40 ft.) boats to a 65 ft. vessel, the R/V Holton

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: Laboratory sections that specialize in aquatic toxicity; organic contaminant analysis; metal analysis; water quality analysis; field environmental assessment; biological monitoring (fish, benthos, phytoplankton, zooplankton) and research; biotechnology applied to environmental toxicity; and fisheries modeling emphasizing survival and growth studies of juvenile fishes.

Major equipment available:

electron microscopy:	JE01, 100CXII SEM/TEM with Kevex Delta-1 x-ray microanalysis system
	Cambridge S100 SEM
spectrophotometry:	2 Finnegan quadrapole GC/MS
	2 Perkin-Elmer 5100 AA spec.'s with graphite furnaces
	1 Thermo-Jarrell Ash video 12 AA spec.'s with graphite furnace
	1 Applied Research Laboratories 3410 ICAP
	1 Perkin-Elmer Model 552 dual beam UV/VIS spectrophotometer
	1 Perkin-Elmer Lambda I UV/VIS single beam spectrophotometer

	2-channel SCI Autoanalyzer system
chromatography:	4 automated GC systems with detectors (3 FID, 3 RCD, 1 FPP, 1 PID)
	1 Waters automated HPLC system (UV and fluorescence)
	1 GPC system
	1 Carlo-Erba NA 1500 carbon/nitrogen analyzer
	1 OI carbon analyzer

Additional Resources

Computerized information retrieval services available:

- Aquatic Biology and Fisheries Abstracts on CD Applied
- Agricultural & Biological Abstracts on CD Bitnet TOXNET

Computers and software available:

- IBM 3090: SAS on CMS, Pascal, FORTRAN (w/IMSL)
- IBM/PC's and PC clones: PCSAS, Turbo Pascal, Harvard Graphics, Statgraphics

Staff available:

- approximately 50 faculty members (Ph.D. level) working in various areas of marine research at ODU
- 8 senior AMRL staff (M.S.-level supervisor/managerial)
- approximately 30 full-time technicians with degress in oceanography, biology, chemistry, geology and environmental health
- numerous graduate research assistants and undergraduate research assistants (20-30)

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989?

GLP requirements are being reviewed; full compliance is expected within 6-12 months; QA/QC protocols are approved by USEPA Bay Program VWCB Safe Drinking Water Program; and independent review (EA) is conducted.

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Aqua Survey, Inc.

Ken Hayes
499 Point Breeze Road
Flemington, New Jersey 08822
201-788-8700
FAX 201-788-9165

Testing site: Aqua Survey, Inc.
499 Point Breeze Road
Flemington, New Jersey 08822

Hours: 8:00 AM - 5:00 PM

Bioassays Conducted

Types of bioassays: Aqua Survey, Inc. (ASI) conducts acute and chronic bioassays using freshwater, estuarine, and marine species of fish, invertebrates, and algae. They also conduct toxicity reduction bioassays and ocean disposal, oil dispersant, and Microtox tests. Some tests may be run in the mobile lab or *in situ*.

Other bioassay work: ASI is equipped with three mobile labs, 14 diluter systems, and has extensive acute, chronic, bioaccumulation (sediments) and TRE
experience.

Future plans: ASI is in the process of patenting a procedure that can be utilized to determine rate and level of toxicity to mixed microorganism populations.

Field sampling capabilities: grab samplers, composite samplers, piston corer, Ekman dredge, Alpha bottles, Kemmerer sampler, zooplankton nets, epibenthic sled, seines

Analytical Chemistry Facilities in Support of Bioassay Procedures
Major area of work or specialty: wet chemistry

Major equipment available:
electron microscopy: none reported
spectrophotometry: none reported
chromatography: none reported

Additional Resources

Computerized information retrieval services available: none reported

Computers and software available: several PC's

Staff available: 1 Ph.D.; 1 M.S.; 7 B.S.'s; 1 B.A.; 1 A.S.; 2 with degrees in progress

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Biological Monitoring, Inc.

Mark Collins, Laboratory Manager
P.O. Box 184
Blacksburg, Virginia 24063
703-953-2821
FAX 703-382-6090

Testing site: Biological Monitoring, Inc.
Route 460 South
Blacksburg, Virginia 24060

Hours: 8:30 AM - 5:30 PM
7 days/week

Bioassays Conducted

Types of bioassays: Biological Monitoring, Inc. (BMI) conducts acute and chronic bioassays on fish, invertebrates, and some algae. (See tables for description of exact test capabilities.)

Other bioassay work: BMI also works with the following species under most test and flow conditions: small mouth bass, striped bass, channel catfish, mummichog, mayflies, stoneflies, caddisflies, amphipods, water penny, blue mussell. Most of BMI's experience with sediments has been testing of elutriates (freshwater and marine). Solid phase studies have been performed in-house but not for contract.

Future plans: BMI plans to expand marine and estuarine culturing and testing capabilities and to increase solid phase sediment testing capabilities.

Field sampling capabilities: 16' outboard motorboat, Kemmerer bottle, Ponar dredge, Surber sampler, kick-nets, plankton nets, field water quality meters, phytometers; experience with freshwater and estuarine biological and chemical surveys

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: standard physiochemical parameters including DO, pH, conductivity, salinity, temperature, alkalinity, hardness, chlorine

Major equipment available:

electron microscopy:	none in house; handled through sub-contract
spectrophotometry:	none in house; handled through sub-contract
chromatography:	none reported

Additional Resources

Computerized information retrieval services available: Chemical Abstracts, Biological Abstracts, Toxline, Toxnet, AQUIRE

Computers and software available: IBM PC/AT's; IBM PS/2 Model 30/286; Toxstat and data system; EPA toxicity test data analysis programs; WordPerfect; Harvard Graphics; Lotus 1-2-3; dBase III; IBM mainframe at Virginia Tech; SAS

Staff available: full-time: 3 Ph.D.'s; 7 B.S.'s; 2 A.S.'s
part-time: B.S.; 5 students; 1 electrical engineer

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Bionetics Corporation, Analytical Laboratories Division

Peter T. Pohorence	Testing site:	Bionetics Analytical Laboratories
20-A Research Drive		20-A Research Drive
Hampton, Virginia 23666		Hampton, Virginia 23666
1-800-476-5548	Hours:	8:00 AM - 5:00 PM
804-865-0880 FAX 804-865-7597		Monday - Friday

Bioassays Conducted

Types of bioassays: Through a joint association with Coastal Bioanalysts of Gloucester Point, VA, Bionetics is able to conduct acute and chronic bioassays of both fish and invertebrates. They also conduct algal growth tests, Microtox tests, Ames mutagenicity tests and others. Note that bioassay testing is not done by Bionetics staff; they only conduct chemical testing. (See tables for complete descriptions of testing capabilities.)

Other bioassay work: Through a joint association with Coastal Bioanalysts, Bionetics offers in-stream impact studies: freshwater, marine, benthic, nekton, phytoplankton, zooplankton, ambient toxicity. They also conduct municipal and industrial Toxicity Reduction Evaluations and develop site-specific water quality criteria/standards.

Future plans: Through a joint association with Coastal Bioanalysts, Bionetics expects to increase the size of its physical plant within the next two years -- modifying to permit freshwater and marine flow-through/bioconcentration studies. Increased capacity for static/static renewal testing is also expected within a year. Another possibility is expansion to mobilize lab capabilities.

Field sampling capabilities: 4 24-hour discrete/composite 1500 field samplers; assorted grab sample apperatus; seine nets. Core samplers, Ponar, Smith/Mac, Peterson, Ekman, Surber Samplers and plankton nets are available by lease.

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: inorganic, metals, and organic chemistry

Major equipment available:

electron microscopy:	none
spectrophotometry:	1 VG Trio GC/MS
	1 Perkin-Elmer 3939 with graphite and auto sampler
	1 IL S-12 with graphite
	1 J-Y 24 Sequential ICP with auto sampler
chromatography:	3 Varian GC's: (2) Model 3300, (1) Model 3400 with auto sampler
	1 Waters GC with auto sampler
	3 Tekmar Purge and Traps
	Detectors available: 2 FID, 4 ECD, 2 PIO, 1 HALL, 1 FPD

Additional Resources

Computerized information retrieval services available: GC/MS database and library search for 48,000 compounds; GC data retrieval system; ICP data retrieval system; DIALOG availability possible

Computers and software available: CLP program data packages for GC/MS, GC, ICP. Novell Data System with 12 workstations. Programs: dBase, Lotus, WordPerfect. (See also Coastal Bioanalysts.)

Staff available: full-time: 1 Ph.D.; 2 M.B.A.'s; 8 B.S.'s; 1 A.A.; 10 non-degree
part-time: 11 non-degree
(See also Coastal Bioanalysts.)

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes* Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

*Coastal Bioanalysts -- Protocol for effluent biomonitoring has been submitted and approved by Virginia Water Control Board. SOP'S submitted to Maryland to date have been approved.

Center for Environmental Studies

Virginia Polytechnic and State University
David R. Orvos
Derring 1020
(VPI&SU)
Blacksburg, Virginia 24061
703-231-5538
FAX 703-231-9307

Testing site: VPI&SU
Derring labs 1027,1027A,1014A,
1020A, 2006
Blacksburg, Virginia 24061

Hours: 8:00 AM - 5:00 PM
(Ecosystem Simulation Labs have
no regular hours.)

Bioassays Conducted

Types of bioassays: The Center for Environmental Studies (CES) is capable of almost any bioassay as prescribed by the contractor.

Future plans: They plan to continue as at present.

Field sampling capabilities: They routinely collect from lakes, streams, wetlands, and rivers.

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: routine water chemistry; soil and wetlands analyses on site

Major equipment available:

electron microscopy:	Both TEM and SEM at 2 sites on campus; includes EDAX.
spectrophotometry:	AA, UV/VIS on site (CES labs)
	MS/GC, ICP on campus
chromatography:	LC in CES lab
	GC, MS/GC on campus

Additional Resources

Computerized information retrieval services available: All commonly used ones.

Computers and software available: IBM and compatibles; 80288-based; Lotus, Word, etc.; IBM 3090 mainframe with SAS, Script, etc.

Staff available: 5 full-time Ph.D.'s; 8 graduate students; 1 research specialist

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? not reported

Follow study protocols? Yes

Have a complete set of Standard Operating Procedures? Presently completing

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Mainframe, yes

Chesapeake Biological Laboratory

University of Maryland, Center for
Environmental and Estuarine Studies
Jay W. Gooch
P.O. Box 38
Solomons, Maryland 20688
301-326-4281
FAX 301-326-6342

Testing site: Chesapeake Biological Lab
UMCEES
Solomons, Maryland 20688

Hours: all

Bioassays Conducted

Types of bioassays: Chesapeake Biological Laboratory has three investigators who do various kinds of cellular biochemical toxicology using aquatic organisms. They are equipped and capable of doing a variety of sublethal biomarker-type assays on Chesapeake Bay organisms. They are capable of doing many others as needs or opportunity allow. (See tables for description of exact testing capabilities.)

Future plans: CBL plans to investigate solid phase sediment bioassays (also pore water, etc.); factors related to equilibrium partitioning; and adaptation to larval fish.

Field sampling capabilities: full range, including research vessels

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: some work in electron microscopy, spectrometry, and chromatography

Major equipment available:

electron microscopy:	SEM
spectrometry:	2 GC/MS
	alpha, beta, gamma counting
	2 AA's
	UV-VIS
	fluorescence
chromatography:	GC
	HPLC
	TLC
	CC
	IC, etc.

Additional Resources

Computerized information retrieval services available: full range of PC's; Macintoshes; DEC-VAX; library with full search capabilities

Computers and software available: full complement of contemporary software-chromatography data systems, etc.

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989?

Not necessarily; CBL is a typical academic research laboratory doing research which is not used for regulatory purposes.

Follow study protocols? generally

Have a complete set of Standard Operating Procedures? some do; some do not

Archive facility for the data generated? Yes

Computer-generated data? Yes

Coastal Bioanalysts, Inc.

Peter F. De Lisle or Ruth L. Williams
P.O. Box 626
Gloucester Point, Virginia 23062
804-642-0168

Testing site: Coastal Bioanalysts, Inc.
Gloucester Point Office Plaza,
Unit C
Route 17
Gloucester Point, Virginia 23062

Hours: 8:00 AM - 4:30 PM
daily

Bioassays Conducted

Types of bioassays: Coastal Bioanalysts, Inc. (CBI) conducts acute and chronic bioassays with both fish and invertebrates. (See tables for descriptions of exact test capabilities.)

Other bioassay work: CBI also performs field impact studies in both freshwater and marine environments for benthos, plankton, and nekton. Ambient toxicity is addressed. Toxicity Reduction Evaluations, both municipal and industrial, are performed. The lab also develops site-specific water quality criteria/standards.

Future plans: CBI expects to increase the size of its physical plant within the next two years. Modifications necessary to permit flow-through/bioconcentration studies (freshwater & marine) are planned at that time. In addition, increased capacity for static/static renewal tests will be available within the next year. If the need arises, addition of a mobile lab for on-site testing is possible.

Field sampling capabilities:
Water: 4 ISCO composite samplers, 2 equipped for Priority Pollutants (at Bionetics)
Sediment: coring devices, samplers: Ponar, Smith-Mac, Peterson, Ekman (leased)
Organisms: Surber sampler, seine net, plankton nets (leased)

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: Through a joint association with the Bionetics Corporation, CBI can perform analyses for organics, inorganics and metals in tissue, water, wastewater, sediment and soil samples. Full GC/MS, ICP, GC, AA, and TOX capability is provided.

Major equipment available:

electron microscopy:	none
spectrophotometry:	1 VG Trio GC/MS with full library search & retrieval capability
	1 Perkin-Elmer Model 3939 AA with graphite furnace
	1 IL S12 AA with graphite furnace
	1 JY 24 Sequential ICP with autosampler
	CLP interface program for GC/MS and ICP
	VG 4 station network data system for above
chromatography:	2 Model 3300 Varian GC
	1 Model 3400 Varian GC with autosampler
	1 Water GC with autosampler
	3 Tekmar Purge and Trap devices

Detectors: 2 FID, 4 ECD, 2 PID, 1 Hall, 1 FPD
CLP program interface for GC's

Additional Resources
Computerized information retrieval services available: Acquisition of public-access online data bases (e.g. DIALOG) is possible.

Computers and software available: PC's, modem; EPA software for calculation of EC50's, probit analysis, analysis of variance and other statistical software; word processing, data management packages.

Staff available: full-time: 2 Ph.D.'s; 1 B.S.; 1 B.A.
part-time: 1 non-degree
(See also Bionetics Corp.)

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

In addition, this lab meets the requirements of NPDES biomonitoring program; e.g., reference toxicity tests are performed on routine basis. Through a cooperative agreement with the Bionetics Corporation, CBI can also provide state certified (MD, VA, NC) chemical analyses and GLP chemical support of TSCA/FIFRA work.

Follow study protocols? Yes* Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? No**

*Protocols for effluent biomonitoring are approved by Virginia Water Control Board. All protocols submitted to date in Maryland have also been approved.

**Computer systems of Bionetics Corp. (providing chemical support) are validated.

Commonwealth Laboratory, Inc.

Edwin Cox III
2209 E. Broad Street
Richmond, Virginia 23223
804-648-8358
FAX 804-644-5820

Testing site: Commonwealth Laboratory, Inc.
305, 307 N. 26th Street
Richmond, Virginia
Hours: 8:30 AM - 5:00 PM
7 days/week

Bioassays Conducted
Types of bioassays: Commonwealth Laboratory, Inc. (CLI) conducts acute and toxic bioassays of dapnia and fathead minnows. (See tables for description of exact test capabilities.)

Future plans: CLI hopes to conduct in situ toxicity testing in 1990.

Field sampling capabilities: flow and time proportional samplers; depth samplers; bottom samplers

Analytical Chemistry Facilities in Support of Bioassay Procedures
Major area of work or specialty: water, wastewater, solids, air

Major equipment available:

electron microscopy:	none
spectrophotometry:	Extrel GC/MS
	2 IL AA 's with furnace
chromatography:	Tracor GC
also:	TOC, TOX, IR, UV and all analyses required by USEPA *except* radiological
	NTIS-certified for bulk asbestos identification

Additional Resources
Computerized information retrieval services available: some

Computers and software available: 1 Epson; 3 KAYPRO 286i

Staff available: full-time: 1 M.Ch.E.; 7 B.S.'s

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989?

No; the lab is in accordance with USEPA Waste Water and Drinking Water, but is not specifically inspected for TSCA and FIFRA.

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Do you have an archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? No

EA Engineering, Science, and Technology, Inc.

Wayne L. McCulloch
EA Mid-Atlantic Regional Operations
Hunt Valley/Loveton Center
15 Loveton Circle
Sparks, Maryland 21152
301-771-4950
FAX 301-771-4204

Testing site: EA Engineering, Science, & Tech.
15 Loveton Circle
Sparks, Maryland 21152

Hours: business: 8:30 AM - 5:00 PM
Monday - Friday
operating: 8:00 AM - 7:00 PM
daily

Bioassays Conducted

Types of bioassays: EA Engineering conducts acute and chronic bioassays on fish, invertebrates and plants. (See tables for description of exact testing capabilities.)

Other bioassay work: EA Engineering can perform Toxicity Identification Evaluations (TIE's) to identify the sources and types of toxicants. They also use bioassays to assist in the development of design-treatability studies for wastewater treatment plants (WWTP's), and can provide consulting services, regulatory interactions and permit review.

Future plans: EA Engineering plans to increase capabilities to perform more TIE/TRES studies, to develop better GLP facilities to provide such services to clients requiring these tests, and to increase consulting facilities.

Field sampling capabilities:

Water: McNeils, Mannings & Iscus composite samplers; metering pumps; Van Dorns or Nishen bottles (for samples at various depths); trash pumps (for large volume water sampling)

Sediment and organisms: Ponar; Ekman; Hess samplers; various nets, seines, and trawls; electroshocking; boats and prams

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: standard priority pollutants; hazardous waste; GC/MS; HPLC's; CLP analyses

Major equipment available:

electron microscopy:	none
spectrophotometry:	6 GC/MS
	4 Tekmar Purge and Trap devices
	1 ICP/AA spectrophotometer
	1 AA spec.
	2 UV/VIS spec.'s
chromatography:	5 GLC
	various detectors
	1 HPLC
	1 GPC

Additional Resources
Computerized information retrieval services available: DIALOG, National Library of Medicine, Dialcom, Chemical Information Systems, Storit

Computers and software available: mainframe VAX and mini-VAX; 5 IBM PC's; 8 laser printers; Perkin-Elmer hardware (LIMS, CLAS); Word II; Word Perfect; SAS; Lotus 1-2-3; Symphony; dBase; Toxstat; Toxcalc; EPA acute and chronic calculation programs, etc.

Staff available: 4 Ph.D.'s; 5 M.S.'s; 1 M.E.M.; 2 B.A.'s

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Environmental Laboratories, Inc.

Steven Pond
9211 Burge Ave.
Richmond, Virginia 23237
804-271-3440
FAX 804-271-1313

Testing site: Environmental Laboratories, Inc.
9211 Burge Ave.
Richmond, Virginia 23237

Hours 24 hrs./day

Bioassays Conducted

Types of bioassays: Environmental Laboratories, Inc. does not conduct bioassays; they provide chemical analysis of environmental samples only.

Future plans: analytical chemistry only

Field sampling capabilities: two full time experienced field services personnel; equipment and personnel for sampling water wastewater, soils, sediments

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: chemical analysis of soils, sediments, water and wastewater for inorganics, organics, trace metals, etc.

Major equipment available:

electron microscopy:	none; but do have polarized light microscopy and plain light microscopy with phase contrast
spectrometry:	1 Perkin-Elmer 2830 AA with furnace
	1 Perkin-Elmer 5000 AA with furnace
	2 each Hewlett Packard 5890 GC's with 5970 MS
	1 Perkin-Elmer 8500 with HALC and PID detectors
chromatography:	2 Hewlett Packard GC's: 5830, 5710
	1 Waters HPLC

Additional Resources

Computerized information retrieval services available: Laboratory Information Management System (LIMS); radian SAM program which tracks samples, accumulates QC and sample data

Computers and software available: 15 IBM PC XT and AT computers; Compaq 286; and Zenith portable with 20 meg hard drive

Staff available:
- 10 chemists
- 6 biologists
- 1 microbiologist
- 5 support

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? not reported

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Environmental Resources Management, Inc.

Robert L. Dwyer, Ph.D.	Testing site:	ERM, Inc.
116 Defense Highway		855 Springdale Drive
Suite 300		Exton, Pennsylvania 19341
Annapolis, Maryland 21401	Hours:	8:00 AM - 5:00 PM
301-266-0006		Monday - Friday
FAX 301-266-8912		additionally by appointment

Bioassays Conducted

Types of bioassays: Environmental Resources Management, Inc. (ERM) conducts acute and chronic bioassays with both fish and invertebrates. They also conduct algal growth studies and Microtox assays.

Other bioassay work: ERM can conduct on-site bioassays (setting up labs at clients' facilities) and ambient toxicity assays (caging sutdies).

Future plans: ERM will be gearing up to do bioassay testing as it becomes necessary for NPDES DMR monitoring. They will also focus on updates of TIE/TRE protocols released by EPA.

Field sampling capabilities:

Water:small boat sampling capabilities; stream sampling capabilities

Benthos and sediment: Ponar grab, diver-operated cores

Fish: seines, gill nets and electroshock equipment

Plankton: bongo net

(All Chesapeake Bay sampling is coordinated and staged from Annapolis office; biology lab in Exton is equipped and staffed for all taxonomic identification.)

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: toxics in the aqueous and solid media; support of activities under CERCLA, RCRA, WQA of 1987, as well as state regulations; industrial hygiene and occupational health analyses

Major equipment available:

electron microscopy:	none at present; planning to add TEM/SEM in 1990
spectrophotometry:	flame and furnace AA's with ICP
	total organic carbon analysis
	visible wavelength spectrophotometer
	Fourier transformed infrared analyzer
chromatography:	3 GC's
	2 GC/MS's
	total halogen analyzer

Additional Resources

Computerized information retrieval services available: complete library retrieval services available (e.g. DIALOG, BIOSIS); access to special databases (CHEMFATE) and government databases (USGS, WATERNET, NAWDEX, EPA-REACH, GEMS)

Computers and software available: Macintosh SE's and II's linked using Applenet. All have Excel, word processors, and MacDraw. 286/386 PC's for statistics, large DBMS. Modeling capabilities include most EPA-approved packages (ERL Athens Center for Exposure Assessment Modeling, International Ground Water Modeling Center, UNAMAP air models). Remote access to mainframe/mini's for SAS, large databases, etc.

Staff available:
Annapolis: 2 Ph.D.'s; 1 M.S.; 2 B.S.'s
Exton, PA: 2 M.S.'s; 1 B.A.; 1 B.S. Also approximately 60 field professionals in these two locations.

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989?

No, neither lab has had an independent GLP audit. Managers believe themselves to be generally in compliance with GLP and could set up easily to do tests for TSCA PMN's and FIFRA product registrations.

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Additional explanation: Computer data storage/retrieval usually customized to meet needs of client. Systems can be designed by subsidiary (ERM Computer Service, Inc.) to comply with CERCLA CLP requirements or TSCA CBI data security protocols as needed.

Environmental Systems Service, Ltd.

Dennis T. Brown, Lab Manager
218 N. Main St.
Culpeper, Virginia 22701
703-825-6660
FAX 703-825-4961

Testing site: Environmental Systems Service, Ltd.
218 N. Main St.
Culpeper, Virginia 22701

Hours: 8:00 AM - 5:00 PM
Monday - Friday
(analysts Saturday and Sunday)

Bioassays Conducted

Types of bioassays: Environmental Systems Service, Ltd. conducts acute and chronic bioassays on fish, invertebrates and algae. (See tables for description of exact testing capabilities.)

Field sampling capabilities: auto samplers, sediment grab samplers, macro-invertebrate bottom samplers

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: chemical analysis of water and wastewater; microbiological analysis of same; food/dairy analysis. Certified in VA and MD.

Major equipment available:

electron microscopy:	none
spectrophotometry:	furnace and flame AA spectrophotometer
	GC/MS
chromatography:	GC
	HPLC (IC)
	TLC

Additional Resources

Computerized information retrieval services available: none reported

Computers and software available: IBM PC's; Wang system; in-house lab-tracking system; Lotus 1-2-3; dBase, Paradox

Staff available: 4 M.S.; 5 B.S.; 1 A.S.; 1 high school

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Experimental Pathology Laboratories, Inc.

Dr. Marilyn J. Wolfe	Testing site:	Experimental Pathology Lab., Inc.
P.O. Box 474		22866 Shaw Road
Herndon, Virginia 22070		Sterling, Virginia 22170
703-471-7060	Hours:	8:00 AM - 5:00 PM
FAX 703-471-8447		Monday - Friday

Bioassays Conducted

Types of bioassays: Experimental Pathology Laboratories, Inc. provides pathology services for invertebrate species (e.g. various crustaceans and insects) and for finfish.

Futher description of bioassay work: EPL's services include necropsy of animals, preparation of tissues for histologic sections, preparation of glass slides for light microscopy, preparation of tissues for electron microscopy and pathologic evaluation of tissues. EPL has processed fish of various ages (larvae through adults) and a variety of species including fathead minnows, bluegill sunfish, green sunfish, striped bass, channel catfish, sheepshead minnows, shad, largemouth bass, Japanese medaka, guppies, goldfish and northern pike. Invertebrate species that have been processed include grass shrimp and mayfly nymphs. Turnaround time from receipt of tissues to a complete report is variable depending upon the size and number of specimens. Services include consultation for the planning of toxicologic studies with aquatic species under controlled laboratory conditions of surveys or aquatic species in natural waterways.

Future plans: EPL is expanding its expertise in invertebrate pathology to include various species of mollusks and other crustaceans.

Field sampling capabilities: EPL will provide pathologists and/or technical staff to perform necropsies on animals in the field and to preserve tissues for histopathology.

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: not reported

Major equipment available:

electron microscopy:	Hitachi 12A transmission electron microscope is available for examination of animal tissues.
spectrophotometry:	none
chromatography:	none

Additional Resources

Computerized information retrieval services available: none

Computers and software available: EPL has a computer system in which histopathology data generated by the pathologist is stored and processed for reporting purposes. The computers are IBM-compatible.

Staff available: 1 Ph.D.; 1 M.S.

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

Is it in compliance with the Good Lab Practices requirements under the Toxic Substances Control Act and the Federal Insecticide, Fungicide, and Rodenticide Act of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes (EPL also has a residual materials archive facility.)

Computer-generated data? Yes; data is generated by a pathologist and entered into a computer.*
Are these computer systems validated? Yes

*Occasionally a study that is done by EPL requires morphometry. Measurement data is computer-generated in that the computer may calculate an area, e.g.,
from a perimeter outlined by a technician on a microscope slide or a photomicrograph.

Free-Col Laboratories, Inc.

William R. Osman
P.O. Box 557
Cotton Road
Meadville, Pennsylvania 16335
814-724-6242
FAX 814-333-1466

Testing site: Free-Col Laboratories, Inc.
Cotton Road
Meadville, Pennsylvania 16335

Hours: 7:00 AM - 9:30 PM
Monday - Friday
(7 days/wk for some bioassay work)

Bioassays Conducted

Types of bioassays: Free-Col Laboratories conducts acute and chronic bioassays with both fish and invertebrates. They also conduct Microtox bacterial toxicity tests. (See tables for more complete descriptions of bioassay conditions.)

Future plans: EPA Test Method 1003.0 algal(Selenastrum capricornutum) growth test

Field sampling capabilities: 3 ISCO autosamplers
3 American Sigma autosampler
3 Wildco Kemmerer samplers
1 Wildco bottom sampler
10 artificial substrates
2 Ekman dredges

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: Free-Col Laboratories, Inc. is a full service independent testing facility specializing in analytical services for environmental protection, occupational health, industrial hygiene, food processing and process quality control. Areas of focus are RCRA and Superfund analyses, technical services, industrial hygiene, hazardous waste and groundwater monitoring, wastewater and drinking water analyses and toxicity (bioassay) testing for clients nationwide and overseas.

Major equipment available:

electron microscopy:	none reported
spectrophotometry:	GC/MS: Finnigan Model 4000, Finnigan Model 5100, Hewlett-Packard Model 597 MSD AA: Perkin-Elmer Model 360, Perkin-Elmer Model 3030, Perkin-Elmer Model 5500, Perkin-Elmer Model 5100/Zeeman
chromatography:	GC: Perkin-Elmer Model Sigma 3B, Perkin-Elmer Model 8700, 2 Hewlett-Packard Models 5880A HPLC: Waters Model 510 with 430 heater

Additional Resources

Computerized information retrieval services available: none reported

Computers and software available: Perkin-Elmer LIMS/2000 System 3205 CPU; IBM PC's; EPA Dunnetts and Probit; Toxstat from University of Wyoming; Symphony,

WordPerfect

Staff available: not reported

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Hampton Roads Sanitation District

George D. Kennedy
P.O. Box 5000
Virginia Beach, Virginia 23455
804-460-2261
FAX 804-460-2372

Testing site: HRSD Special Projects Lab
1436 Air Rail Avenue
Virginia Beach, Virginia 23455

Bioassays Conducted

Types of bioassays: Hampton Roads Sanitation District (HRSD) conducts acute and chronic bioassays on estuarine fish and invertebrates. (See tables for exact testing capabilities.)

Other bioassay work: HRSD currently conducts NPDES required tests for 3 POTW's. Quarterly testing requirements on each include at least 2 acute and 1 chronic test.

Future plans: HRSD would like to conduct NPDES required tests for 9 POTW's.

Field sampling capabilities: 25' fully-equipped oceanographic research vessel
Loran navigational recording fathometer
submersible pump assembly or NIO bottles
otter trawls, hand seines, and traps
Ponar and Van Veen sediment grabs

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: wastewater treatment process control; industrial waste characterization; metals; nutrients

Major equipment available:

electron microscopy:	none
spectrophotometry:	AA spectrophotometer
	UV/VIS spectroscopy
	auto analyzer colorimetric system

Additional Resources

Computers and software available: IBM-compatibles: HP ES12, Compaq 286's; Macintosh SE's; Lotus 1-2-3; WordPerfect 5.0; Revelation; Statgraphics; SAS; Harvard Graphics; Excel

Staff available:
toxicity: 2 Ph.D's; 3 B.S.'s; 3 A.A.'s
chemistry: 1 Ph.D.; 6 M.S.'s; 2 B.S. or equivalent
statistics/computer: 2 M.S.'s

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Representatives from HRSD have not read FIFRA and TSCA GLP of August 17, 1989. Their QA/QC program was developed based on the
EPA acute and chronic manual and previous GLP's.

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? *

*HRSD has EPA programs to calculate LC's and NOEC's. They do not store data or transfer original lab work sheets onto the computer.

Horn Point Environmental Laboratory

University of Maryland, Center for
Environmental and Estuarine Studies
Ian Hartwell/ Charles Hocutt
P.O. Box 775
Cambridge, Maryland 21613
301-228-8200
FAX 301-476-5490

Testing site: Horn Point Environmental Lab
UMCEES
P.O. Box 775
Cambridge, Maryland 21613

Hours: not reported

Bioassays Conducted

Types of bioassays: Horn Point Environmental Lab conducts extensive acute and chronic bioassays on fish, invertebrates, and various algae, for all salinity ranges. (See tables for exact test capabilities.)

Future plans: none beyond those described

Field sampling capabilities: seines, trawls, trap nets, dip nets, etc.; ISCO water samplers; bottom dredge

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: effluents -- basic water chemistry and autoanalyser

Major equipment available:

electron microscopy:	none
spectrophotometry:	flame AA spec.
	4 MS's
chromatography:	3 GC's
	IC
	2 HPLC's
detectors:	TCD, 2 FID, EC

(Above equipment is primarily dedicated to nutrient analysis.)

Additional Resources

Computerized information retrieval services available: DIALOG

Computers and software available: VAX 780; VAX 750; Micro VAX; IBM PC's; access to supercomputing

Staff available: 2 Ph.D.'s; 2 M.S. students

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? No

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? No

Archive facility for the data generated? No

Computer-generated data? No

James R. Reed and Associates

Elaine Glover or Liz Christoff
813 Forrest Drive
Newport News, Virginia 23606
804-599-6750
FAX 804-591-7680

Testing site: James R. Reed and Associates
813 Forrest Drive
Newport News, Virginia 23606

Hours: 8:00 AM - 5:00 PM
Monday - Friday

Bioassays Conducted

Types of bioassays: James R. Reed and Associates conducts acute and chronic bioassays with both fish and invertebrates. They also perform algal growth tests. (See tables for exact test capabilities.)

Other bioassay work: In addition to routine bioassay work, this facility is experienced in conducting site-specific studies and developing protocols for
indigenous (non-standard) test organisms.

Future plans: James R. Reed and Associates plans to begin conducting bioassays in association with Toxicity Identification Evaluations and Toxicity Reduction Evaluations for NPDES dischargers within the next year. Within the next three years, the lab plans to develop toxicological capabilities for sediment analysis and begin operation of a mobile laboratory unit.

Field sampling capabilities:

Water: grab; 24-hr. composite and/or 7-day composite water sampling with Isco pump; groundwater monitoring using bailers or dedicated tubing; subsurface water collection using Isco pump and BETA plus horizontal water sample bottles

Sediment: Ponar grab; core sampling; dredging; extraction of water from soil using a lysimeter

Macrobenthic sampling: D-frame nets

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty:

- wastewater/NPDES dischargers (priority/non-priority pollutants)
- groundwater monitoring
- drinking water scans
- soils analysis
- landfill leachates

Major equipment available:

electron microscopy:	none
spectrophotometry:	Finnigan GC/MS
Varian AA spec.	(flame and graphite furnace)
	Beckman spectrophotometer
chromatography:	Varian GC
	ECD, FID detectors

Additional Resources
Computerized information retrieval services available: Toxicological Network (TOXNET) available 24-hr.

Computers and software available: 6 IBM PC-compatible computers with Multimate word processor, Quatro spreadsheet and graphics and Toxistat/EPA statistical programs

Staff available: 1 Ph.D; 2 M.S.'s; 12 B.S.'s

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989?

This lab is in compliance with GLP standards under the Toxics Substances Control Act of November 29, 1983 with the following execption: due to the small size of the laboratory, it has no in-h
ouse chain of custody documentation other than a labsheet which has all required information.

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? No

Additional explanation: Computer printout is validated for computer-generated data, and hand calculations are available in SOP's.

Johns Hopkins University

Applied Physics Lab	Testing site:	Johns Hopkins University
Dennis T. Burton		Applied Physics Laboratory
4800 Atwell Road		4800 Atwell Road
Shady Side, Maryland 20764		Shady Side, Maryland 20764
301-867-7000	Hours:	8:30 AM - 5:00 PM
FAX 301-867-0839		Monday - Friday
		Variable hours Saturday and Sunday

Bioassays Conducted

Types of bioassays: The JHU/APL Toxicology and Bioassay Facility can conduct the following types of aquatic bioassays: acute, short-term chronic, partial life stage, early life stage (ELS) and full life cycle tests. (See the Applied Physics Lab row in the attached tables for more specific information about these bioassays.)

Other bioassay work: Several estuarine and freshwater fish, invertebrates, and algae are maintained in culture at the facility. The laboratory can perform toxicity tests with contaminants in water, sediments, and groundwater, as well as media with toxic volatile compounds. JHU/APL performs all of the acute and short-term chronic tests for the Maryland Department of the Environment NPDES permits compliance division, and has produced two manuals: "Standard Operating Procedures for Acute Effluent Toxicity Tests with Freshwater and Saltwater Organisms" and "Standard Operating Procedures for Short-Term Chronic Effluent Toxicity Tests with Freshwater and Saltwater Organisms."

Future plans: The JHU/APL Toxicological and Bioassay Facility located in Shady Side, Maryland, will be moving during the summer of 1990 from JHU/APL to the University of Maryland Agricultural Experiment Station Wye Research and Education Center, Queenstown, Maryland. The move will increase our present toxicity testing space from 2,000 to 3,000+ sq. ft. Chemistry laboratory space will be increased from 900 sq. ft. to ~1,600 sq. ft. at the Wye Center.

field sampling capabilities:

Water: Niskin bottles (single or Rosette), Kemmerer samplers, pumping, etc.; rotating disk and flat plate samplers are used to sample surface microlayers.

Organisms: boats, seines, push nets, plankton nets, traps, artificial substrates, Hess samplers, Surber samplers and other devices

Sediments: single and multiple core samplers, Ekman dredges, Kellen grab, Ponar grab, etc.

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: heavy metals, several classes of organics, nutrients

Major equipment available:

electron microscopy:	none
spectrophotometry:	Perkin-Elmer AA Model 2380 equipped with an HGA 400 graphite furnace and MHZ-200 mercury-hydride system

chromatography:	Waters Associates HPLC Model 680 controller equipped with a Model 481 variable wavelength UV spectrophotometer Model 740 data integrator; and Model 712 WISP automatic sampler Hewlett Packard GC Model 5890 equipped with flame ionization and flame photometric detectors and an HP 3393 A integrator and HP 7673 automatic sampler (An IBM-AT PC with Interactive Microware software is used for HPLC and GC control and sample analysis.)

Additional Resources

Computerized information retrieval services available:

- Access via PC modem to the DIALOG Inc., the Orbit System, BRS, and DOE-RECON Literature Search.
- DIALOG and Orbit Systems provide access to over 350 other data bases which include the NTIS and Dissertation Abstracts.
- Other data bases that can be accessed are BIOS Previews from Biological Abstracts Inc., Chemical Abstracts Service, Oceanic Abstracts, Pollution Abstracts, Environline, EMBASE, Water Resources Abstracts, Aquatic Science and Fisheries Abstracts, etc.

Computers and software available:

- several IBM AT PC's
- WordPerfect 5.0, Symphony 2.0, Lotus 1-2-3, Sigmaplot 3.1, Norton's Utility Microsoft Chart 3.0, PC SAS, Toxstat 2.0, EPA Bioassay Statistical Program, etc.
- access via modems to the IBM mainframe computers at the Applied Physics Laboratory which contain many software programs, programming languages, and graphic art capabilities

Staff available: 3 Ph.D.'s; 1 M.A.; 2 M.S.'s; 3 B.A.'s; 3 B.A.'s

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Malcolm Pirnie, Inc.

Jane Hughes or Meryl Alexander
100 Grasslands Road
Elmsford, New York 10523
914-347-2974
FAX 914-347-2984

Testing site: 100 Grassland Road
Elmsford, New York 10523
(also) 301 Hiden Blvd.
P.O. Box 6129
Newport News, Virginia 23606

Hours: Monday - Friday
8:00 AM - 5:00 PM

Bioassays Conducted

Types of bioassays: Malcolm Pirnie conducts acute and chronic bioassays on fish and invertebrates. (See tables for exact test capabilities.)

Future plans: Malcolm Pirnie may begin microcosm studies.

Field sampling capabilities: Ponar grab sampler, sediment corers, water samplers (Van Dorn, etc.), electroshocker, swines, plankton nets, periphyton samplers

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: analyses of water, wastewater, hazardous waste, sediments, sludges

Major equipment available:

electron microscopy:	none
spectrophotometry:	1 MS
	1 AA spec.
	2 spectrophotometers
chromatography:	1 GC/FID
	1 HPLC/UV detector
	1 GC/ECD
	1 GC/NPD
	1 GC/MS
	1 Purge and Trap device

Additional Resources

Computerized information retrieval services available: DIALOG - includes Aquatic Science and Fisheries Abstracts, BIOSIS, Enviroline, etc.; C15 - includes OHM/TADS, AQUIRE, ENVIROFATE, CHRIS, CESARS, PHYTOTOX, etc.

Computers and software available: LIMS is being implemented for lab; Prime minicomputer with assorted engineering software, including CAD; > 200 personal computers (IBM-compatible) with assorted software for word processing (WordPerfect, PC Write) for spreadsheets (1-2-3) and statistics (SAS, Statgraphics); desktop publishing and graphics

Staff available: full-time: (chemistry) 2 M.S.'s; 3 B.S.'s; 2 A.S.'s
part-time: (toxicology) 1 M.S.; 1 M.S.P.H

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? *

*Computer programs are run with standard data sets; programs and outputs are then part of SOP's.

Olver Incorporated

Robert M. Roberts, P.E.
1116 South Main Street
Blacksburg, Virginia 24060
703-552-5548
FAX 703-552-5577

Testing site: Olver Incorporated
1116 South Main Street
Blacksburg, Virginia 24060

Hours: 8:00 AM - 5:00 PM
Monday - Friday

Bioassays Conducted

Types of bioassays: Olver Incorporated conducts acute and chronic bioassays using both fish and invertebrates. They also perform chronic algal growth tests. (See tables for descriptions of exact test capabilities. Note that the "number of tests our lab can run simultaneously" is based on running one test type only.)

Other bioassay work: Olver Incorporated has the capability and capacity to provide screening and definitive tests using many different species, such as Lepomis machrochirus, Salmo gairdneri, Ictalurus punctatus, and others, simply by obtaining these species from commercial suppliers. Chronic survival and reproduction testing is also possible for D. pulex and D. magna, although methods are not presented for these organisms in the EPA manual EPA/600/4-89/001, March 1989.

Future plans: Facilities are being expanded in order to have more capacity for the same types of testing currently conducted. Within six months they hope to double the number of tests they can perform simultaneously. Olver also plans to expand its capabilities to accommodate Toxicity Reduction Evaluations as well as site-specific studies. They will continue trial seawater tests, with the intent of offering these commercially at some time.

Field sampling capabilities: Olver Incorporated has a full range of field sampling capabilities, including qualified technicians and equipment. Routine collections of water, wastewater, sediment, sludge, soil and hazardous materials/waste samples are made. The laboratory staff also perform macroinvertebrate stream surveys and collect native test organisms.

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: inorganic chemistry; wet chemistry; microbiological tests; treatability studies; Toxicity Reduction Evaluations; EP Toxicity and TCLP extractions

Major equipment available:

electron microscopy:	none
spectrophotometry:	1 Perkin-Elmer 1100 AA spectrophotometer
	1 Perkin-Elmer 305B AA spectrophotometer
chromatography:	1 HP 5730A GC
	1 HP 5780A GC

Additional Resources

Computerized information retrieval services available: none

Computers and software available: IBM System 3600; IBM PC's; Compaq 386; EPA statistical programs for the analysis of toxicological data (LC 50 determination); Toxstat statistical programs for the analysis of toxicological data

Staff available: full-time: 1 M.S.; 2 B.S.'s
part-time: 1 B.S.

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

(This lab is in the process of complying with the Good Lab Practices requirements of TSCA and FIFRA.)

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Patuxent Wildlife Research Center

U.S. Fish and Wildlife Service
Dr. Harry N. Coulombe
Laurel, Maryland 20708
301-498-0279
FAX 301-497-0515

Testing site: Patuxent Wildlife Research Center
U.S. Fish and Wildlife Service
Laurel, Maryland 20708

Hours: 8:00 AM - 4:30 PM
Monday - Friday

Bioassays Conducted

Types of bioassays: The Patuxent Wildlife Research Center conducts autotrophic and microcosm system tests and photosynthesis tests on the sago pondweed, Potamogeton pectinatus.

Further description of bioassay work: The above test systems are in various stages of development. The Wildlife Research Center does not run standard analyses, except for several herbicides used to develop the systems. The three laboratory procedures need to be verified by in situ tests (i.e., floating pods which are currently being tested). The lab systems use synthetic or natural medias and substrates and can be used to test the effects on plant growth and morphology of single chemicals, combinations of chemicals, and other environmental stressors (e.g., light intensity). Large numbers of explants can be propagated (cloned) using axenic techniques.

Future plans: The Patuxent Wildlife Research Center plans to verify tests for the three laboratory procedures as described above and to utilize procedures
in large scale testing.

Field sampling capabilities: two boats: 17' whaler and 21' Roballo; box core sampler; benthic air-lift device; Solomat water quality analyzer

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: analysis of tissue, soil, water, and plant samples for pesticides, metals, and PAH's

Major equipment available:

electron microscopy:	none
spectrophotometry:	MS: 2 routine analysis mass specific detector GC/MS systems (HP); 1 research-grade GC-MS/MS system (Finnigan) AA: 3 Perkin-Elmer graphite furnace systems; 2 flame AA systems and 2 cold vapor mercury analyzers ICP: 1 Perkin-Elmer ICP Arc. system for analyses of inorganic chemicals
chromatography:	GC's: several GC's; packed systems (3) and capillary systems (10) with computer aided data capture (Nelson) and various detectors (PID, FPD, NPD, ECD, and FID) LG: multiple HPLC capabilities with various detectors, UV, VIS, both variable and fixed wavelength, and continuously variable fluorometric conductivity detectors Maxima automated data handling station

Additional Resources

Computerized information retrieval services available: Library has on-line access to two literature data bases -- DIALOG and OCLC.

Computers and software available: IBM-compatible and Apple PC's; Hewlett Packard 3000 minicomputer; Prime 9955-II minicomputer; access via terminals and phone lines to NIH computing facility; variety of statistical, spreadsheet, graphics and word processing packages; programmers also available.

Staff available: 19 Ph.D.'s; 12 B.S.'s; 6 M.S.'s

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

Is it in compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? No

Patuxent's work does not usually involve FIFRA. Consequently, they have not adopted all of the GLP procedures. They feel that the quality of the data they generate is as good or better than that of any facility in full compliance.

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? No*

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? No

*SOP's are being developed.

Riverside Laboratories

Gloria Gibson
1300 Old Denbigh Boulevard
Newport News, Virginia 23602
804-886-3900
FAX 804-886-3988

Testing site: Riverside Laboratories
1300 Old Denbigh Boulevard
Newport News, Virginia 23602

Hours: 24 hours a day

Bioassays Conducted

Types of bioassays: Riverside Laboratories does not report capabilities for standard bioassay work. They do conduct histological and microbiological studies.

Other bioassay work: Riverside Laboratories conducts work on a full range of organisms pathogenic to man.

Future plans: DNA probes for pathogenic organisms.

Field sampling capabilities: none

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: complete analytical biochemistry testing on tissue, blood, urine

Major equipment available:

electron microscopy:	SEM
spectrophotometry:	GC/MS
	Bio Chemical Analyzer
	AA with graphite furnace and cold
	Radio Isotopes Gamma and Beta counter
	ICP
chromatography:	GC

Additional Resources

Computerized information retrieval services available: Yes

Computers and software available: Reporting and statistical work with Cerner system.

Staff available: not reported

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

Technical Testing Laboratories

Mr. Dilip V. Kalyani, P.E.
4643 Benson Avenue
Baltimore, Maryland 21227
301-247-7400
FAX 301-247-7402

Testing site: Technical Testing Laboratories
4643 Benson Avenue
Baltimore, Maryland 21227

Hours: 8 hrs./day
7 days/week

Bioassays Conducted

Types of bioassays: Technical Testing Laboratories conducts acute and chronic bioassays with both fish and invertebrates. (See tables for descriptions of exact test capabilities.)

Other bioassay work: Technical Testing Laboratories is also equipped to perform Microtox toxicity tests.

Future plans: Extensive Toxicity Reduction Evaluations (TRE) and Toxicity Identification Evaluations (TIE).

Field sampling capabilities: grab; 24-hour time proportional; 24-hour flow proportional; sediment sampling

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: inorganic non-metals; inorganic metals with AA, ICP, etc.; air; soil

Major equipment available:

electron microscopy:	none
spectrophotometry:	GC/MS, HPLC, TCLP, AA, ICP, TOX, TOC, FTIR, IR, etc.
chromatography:	GC

Additional Resources

Computerized information retrieval services available: LIMS

Computers and software available: IBM and IBM-compatibles

Staff available (degree status, number, and time): full-time: 2 Ph.D.'s; 2 M.S.'s; 14 B.S.'s; 9 technicians

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

University of Maryland

Agricultural Experiment Station
Lenwood Hall
Wye Research and Education Center
P.O. Box 169
Queenstown, Maryland 21658
301-827-6202
FAX 301-827-9039

Testing site: University of Maryland
Agricultural Experiment Station
Wye Research and Education Center
Queenstown, Maryland 21658

Note: This facility was undergoing reorganization at press time. For most of calendar year 1990, two stations will be maintained -- the previously established Johns Hopkins University Applied Physics lab (see entry by that title) and a new facility developed at the Wye Research and Education Center named above. The capabilities for each lab are listed as identical: contact either laboratory for more information.

Bioassays Conducted

Types of bioassays: The UM Agricultural Experiment Station can conduct the following types of aquatic bioassays: acute, short-term chronic, partial life stage, early life stage (ELS) and full life cycle tests. (See the Applied Physics Lab row of the tables in back for more specific bioassay descriptions.)

Other bioassay work: Several estuarine and freshwater fish, invertebrates, and algae are maintained in culture at the facility. The laboratory can perform toxicity tests with contaminants in water, sediments, and groundwater, as well as media with toxic volatile compounds.

Future plans: The JHU/APL Toxicological and Bioassay Facility located in Shady Side, Maryland, will be moving during the summer of 1990 from JHU/APL to the University of Maryland Agricultural Experiment Station Wye Research and Education Center, Queenstown, Maryland. The move will increase our present toxicity testing space from 2,000 to 3,000+ sq. ft. Chemistry laboratory space will be increased from 900 sq. ft. to ~1,600 sq. ft. at the Wye Center.

Field sampling capabilities:

Water: Niskin bottles (single or Rosette), Kemmerer samplers, pumping, etc.; rotating disk and flat plate samplers are used to sample surface microlayers.

Organisms: boats, seines, push nets, plankton nets, traps, artificial substrates, Hess samplers, Surber samplers and other devices

Sediments: single and multiple core samplers, Ekman dredges, Kellen grab, Ponar grab, etc.

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: heavy metals, several classes of organics, nutrients

Major equipment available:

electron microscopy:	none
spectrophotometry:	Perkin-Elmer AA Model 2380 equipped with an HGA 400 graphite furnace and MHZ 200 mercury-hydride system

chromatography: Waters Associates HPLC Model 680 controller equipped with a Model 481 variable wavelength UV spectrophotometer
Model 740 data integrator; and Model 712 WISP automatic sampler
Hewlett Packard GC Model 5890 equipped with flame ionization and flame photometric detectors and an HP 3393 A integrator and HP 7673 automatic sampler (An IBM-AT PC with Interactive Microware software is used for HPLC and GC control and sample analysis.)

Additional Resources
Computerized information retrieval services available:
- Access via PC modem to the DIALOG Inc., the Orbit System, BRS, and DOE-RECON Literature Search.
- DIALOG and Orbit Systems provide access to over 350 other data bases which include the NTIS and Dissertation Abstracts.
- Other data bases that can be accessed are BIOS Previews from Biological Abstracts Inc., Chemical Abstracts Service, Oceanic Abstracts, Pollution Abstracts, Environline, EMBASE, Water Resources Abstracts, Aquatic Science and Fisheries Abstracts, etc.

Computers and software available:
- several IBM AT PC's
- WordPerfect 5.0, Symphony 2.0, Lotus 1-2-3, Sigmaplot 3.1, Norton's Utility Microsoft Chart 3.0, PC SAS, Toxstat 2.0, EPA Bioassay Statistical Program, etc.
- access via modems to the IBM mainframe computers at the Applied Physics Laboratory which contain many software programs, programming languages, and graphic art capabilities

Staff available: 3 Ph.D.'s; 1 M.A.; 2 M.S.'s; 3 B.A.'s; 3 B.A.'s

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes

University of Maryland at Baltimore
Aquatic Toxicology Facility

Andrew S. Kane and R. Reimschuessel
UM School of Medicine
Department of Pathology
10 South Pine Street
Baltimore, Maryland 21201
(301) 328-7230/7276
FAX (301) 328-8414

Testing site: UM School of Medicine
Department of Pathology --
Aquatic Toxicology Facility
610 West Lombard Street
Baltimore, Maryland 21201

Hours: 24 hours per day
7 days per week

Bioassays Conducted

Types of bioassays: The Aquatic Toxicology Facility conducts both acute and chronic bioassays on fish. (See tables for descriptions of exact test capabilities.)

Other bioassay work: Several flow-through systems are available for bioassay testing and are set up on a demand basis. These include three solenoid-operated EnvironTox dilutors, three all-glass flow-through dosing systems with pH and temperature control, and a 600-gallon polypropylene flow-through dilutor system. There is also an isolated testing room for static or flow through testing. Additional on-site support facilities include histology and electron microscopy laboratories, tissue and organ culture facilities, a diagnostic microbiology laboratory, office space and a library.

Future plans: This facility is planning for expanded necropsy and tissue culture space; two additional isolated testing rooms; and additional office space and a conference room.

Field sampling capabilities: Sampling and collection services are available and are arranged on a per-contract basis.

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: descriptive toxicology; diagnostic and comparative pathology; carcinogenesis assays - in vivo and in vitro

Major equipment available:

electron microscopy:	2 JEOL 100B TEM's
	1 AMR 1000 SEM
	1 JEOL100CX TEM CAN with an ASID attachment used for transmission, scanning transmission and secondary scanning electron microscopy. (All necessary equipment for specimen preparation is present, including a polaron critical point drying apparatus and a Techniques Hummer sputtering device for coating.)
spectrophotometry:	Gilford "Response" automated spectrophotometer
	Farrand ratio fluorometers
	Perkin-Elmer MPF spectrofluorometer
	Abbot TDX Polarized Fluorescence Scanner
chromatography:	Waters HPLC system with two pumps and variable wavelength detector
	Varian AA 575 spec.

	Varian 2700 GC
other:	Corning Model 175 blood-gas analyzer
	Beckman Astra 8, Kodak Ektachem 400 and Ektachrome 700 analyzers (for electrolytes, urea nitrogen, protein, creatinine, enzymes, etc.)
	complete facilities for enzyme and immuno-histochemistry

Additional Resources

Computerized information retrieval services available: Toxline, Medline, MaryMed, BioAbstracts, Zoological Abstracts, Chemical Abstracts, Current Contents

Computers and software available: IBM AT's, Macintosh II's, UMAB Mainframe; variety of word processing and graphics packages, SAS, Lotus, Minitab, Montage

Staff available:

full-time:	1 V.M.D./Ph.D.; 1 Ph.D.; and 1 M.S.
part-time:	3 Ph.D.'s;1 B.A./V.T.; and 3 B.A.'s (some with M.S.)

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? Yes, SAS

University of Maryland, Baltimore County

Brian Bradley
Department of Biological Sciences
Baltimore, Maryland 21228
301-455-2244
FAX 301-455-3875

Testing site: UMBC
Dept. of Biological Sciences
Baltimore, Maryland 21228
Hours: not reported

Bioassays Conducted
Types of bioassays: UMBC does not conduct traditional bioassays.

Future plans: UMBC is aiming to have a rapid field test (based on stress-related protein synthesis) available in the next 1 to 3 years. They also expect to have immuno diagnostic capability to identify specific contaminants (or classes) in mixtures.

Field sampling capabilities: not reported

Analytical Chemistry Facilities in Support of Bioassay Procedures
Major area of work or specialty: molecular/cellular biology

Major equipment available:

electron microscopy:	several TEM
	several SEM
spectrophotometry:	full range: national center for MS
chromatography:	full range

Additional Resources
Computerized information retrieval services available: BIOSIS, Medline, Environline, etc.

Computers and software available: MAC; VAX mainframe; IBM PC's

Staff available: 26 Ph.D.'s in department; one lab with 2 Ph.D.'s and 6 graduate students doing bioassays (biochemical)

Quality Assurance/Quality Control Procedures and Capabilities
QA/QC program? No

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989?

No, but Radiation Safety Committee and Human Hazard Committee oversee all labs.

Follow study protocol? Yes Have a complete set of Standard Operating Procedures? not reported
Archive facility for the data generated? No

Computer-generated data? No

Versar, Inc., ESM Operations

Dr. Steve Weisberg
9200 Rumsey Road
Columbia, Maryland 21045
301-964-9200
FAX 301-964-5156

Testing site: Versar, Inc., ESM Operations
9200 Rumsey Road
Columbia, Maryland 21045

Hours: 8:00 AM - 4:30 PM

Bioassays Conducted

Types of bioassays: Versar conducts acute and chronic bioassays using both fish and invertebrates. They also conduct sediment bioassays andin situ bioassays with juvenile fish. (See tables for descriptions of exact test capabilites.)

Future plans: Versar is currently renovating facility to enlarge holding and testing capabilities.

Field sampling capabilities: Five sampling boats equipped with a complete array of nets, trawls, sleds, corers, grabs, and other sampling equipment. Back-pack and boat-mounted electroshockers, eel pots, minnow traps, crab pots, and seines are also on-hand.

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: Versar's Springfield laboratory is a full service facility with capability to perform organic and inorganic analyses on
priority pollutants in water, sediment, and biota.

Major equipment available:

electron microscopy:	none
spectrophotometry:	GC/MS
	AA
	ICP
	UV-VIS scanning spectrophotometer
chromatography:	GC
	Ion chromatographs
	HPLC

Additional Resources

Computerized information retrieval services available: DIALOG, MEDLARS, CIS systems for retrieval of ecological and toxicological data and literature

Computers and software available: VAX/VMS operating system; PC's -- Lotus, WordPerfect, Graphwriter, SAS, EPA statistical programs for aquatic toxicology; Hewlett Packard plotters

Staff available: 7 Ph.D.'s; 10 M.S.'s; 2 B.S.'s

Quality Assurance/Quality Control Procedures and Capabilities

QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? No

Archive facility for the data generated? Yes

Computer-generated data? Yes Are these computer systems validated? not reported

Virginia Institute of Marine Science

R. Huggett/M. Roberts
VIMS, Division of Chemistry
and Toxicology
Gloucester Point, VA 23062
804-642-7236

Testing site: VIMS, Division of Chemistry and Toxicology Gloucester Point, VA 23062

Hours: not reported

Bioassays Conducted

Types of bioassays: VIMS conducts acute and chronic bioassays on fish and chronic bioassays on invertebrate species. (See tables for description of exact test capabilities.)

Future plans: none beyond those described

Field sampling capabilities: vessels of various sizes; otter trawl nets; Smith MacIntyre grabs

Analytical Chemistry Facilities in Support of Bioassay Procedures

Major area of work or specialty: organic chemicals/varies

Major equipment available:

electron microscopy:	1 TEM
	1 SEM
spectrophotometry:	2 MS
chromatography:	numerous GC
	HPLC

Additional Resources

Computerized information retrieval services available: Hewlett Packard Data System for analytical data reduction

Computers and software available: PC's; Prime 9955

Staff available: 1 Marine Scientist A; 1 Laboratory Specialist A

Quality Assurance/Quality Control Procedures and Capabilities QA/QC program? Yes

In compliance with Good Lab Practices requirements of TSCA and FIFRA of August 17, 1989? Yes

Follow study protocols? Yes Have a complete set of Standard Operating Procedures? Yes

Archive facility for the data generated? Yes

Computer-generated data? partly Are these computer systems validated? Yes

ACUTE FISH BIOASSAYS

LABORATORY	SPECIES	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	HOLDING CAPACITY	TURNAROUND TIME	# RUN AT ONCE
Applied Marine Res. Lab.	*Cyprinodon variegatus*	lab, water, S	< 90 days	96 hr	15-25 ppt	yes	30 days	10
	"	lab, sed., S	same	10 days	"	"	45 days	6
Applied Physics Lab. of Johns Hopkins University, and the University of Maryland Agricultural Experiment Station	*Menidia beryllina*	lab, mob, SR, C	all	4 days	1-15 ppt	yes	5-6 days	4 to 10
	Fundulus sp.	All lab, mobile and in situ, and all SR and C.	"	"	2-15 ppt	"	"	"
	Cyprinodon variegatus		"	"	"	"	"	"
	Morone saxatilis		E, L, J	"	"	"	variable	"
	Alosa aestivalis	"	E, L	"	0-5 ppt	"	"	"
	Alosa sapidissima	"	E, L, J	"	0-5, 5-15	"	"	"
	Perca flavescens	"	"	"	"	"	"	"
	Morone americana	"	L, J	"	"	"	"	"
	Leostomus xanthurus	lab, mob, SR, C	juveniles	"	5-15 ppt	"	"	"
	Brevoortia tyrannus	"	"	"	"	"	"	"
	Trinectes maculatus	"	"	"	"	"	"	"
	Pimephales promelas	Lab, mob and in situ; S, SR, and C.	all	"	0	yes	5-6 days	4 to 10
	Lepomis macrochirus		juv, adult	"	"	"	"	"
	Ictalurus sp.		"	"	"	"	"	"
	Salmo gairdneri	lab, mobile	all	"	"	"	"	"
Biological Monitoring, Inc.	*Lepomis macrochirus* *Pimephales promelas* *Salmo sp.*	all, S, SR, C	all	24-96 hr	0 ppt	yes	2 wks post-test	15
	Cyprinodon variegatus *Menidia sp.*	"	"	"	3-33 ppt	"	"	"
Center for Environ. Studies Virginia Tech.	*Pimephales promelas*	lab, S, SR	as prescribed	as pre-scribed	as prescribed	yes	4-5 days	4 to 6
Coastal Bioanalysts, Inc.	*Cyprinodon variegatus* *Menidia beryllina* *Fundulus heteroclitus* *Pimephales promelas*	lab, S, SR	juvenile	48-96 hr	20-34	yes	1 week	30
	Salmo gairdneri	"	"	"	"	no	"	3
	Lepomis macrochirus	"	"	"	0	"	"	3
Commonwealth Labs, Inc.	*Pimephales promelas*	lab, S		96 hrs	< 30 ppt	yes	2 days	6

acute fish, continued

LABORATORY	SPECIES	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	HOLDING CAPACITY	TURNAROUND TIME	# RUN AT ONCE
EA Engin., Science, Tech., Inc.	*Pimephales promelas* *Lepomis macrochirus* *Salmo gairdneri* *Notemigonus sp.* *Menidia sp.* *Morone saxatilis*	All can be conducted in the laboratory, a mobile lab, or in situ.	< 90 days	96 hrs	20-30 ppt for the saltwater tests	yes	maximum of 30 days from end of test	10
	Leostomus xanthurus *Cyprinodon variegatus*	S, SR, C	"	"	"	"	"	"
Environ. Resources Mgt.	*Pimephales promelas*	lab, SR	< 90 days	96 hr	25-30 ppt	yes	2 weeks	7
	Ictalurus punctatus *Fundulus heteroclitus* *Cyprinodon variegatus*	"	"	"	"	no	"	"
Environ. Systems Service, Ltd.	*Pimephales promelas*	lab, SR	<90 days	48 hr	N/A	yes	10 days	4
Free-Col Laboratories	*Pimephales promelas*	lab, S, SR	<14 days	96 h	0	yes	not given	4
Hampton Roads Sanitation District	*Fundulus heteroclitus*	lab, S	< 90 days	48-96 hr	18-22 ppt	yes	4-6 days	4
	Cyprinodon variegatus	"	"	48 hr	"	"	10 days	2
Horn Point Environmental Lab. University of Maryland	*Pimephales promelas*	lab, S, SR	all	any	any	yes	1 week	"
	Morone saxatilis *Lepomis macrochirus* *Ictalurus punctatus* *Pimephales dentatus* *Fundulus sp.* *Lagodon rhomboides* *Cyprinodon variegatus* *Menidia sp.* *Leiostomus xanthurus* *Gasterosteus aculeatus*	"	egg/ juv	"	"	"	"	"
James R. Reed and Assoc.	*Pimephales promelas*	lab, S	juvenile	96 h	N/A	yes	2 weeks	6
	Cyprinodon variegatus *Fundulus heteroclitus*	"	"	"	20-24	"	"	"
Malcolm Pirnie, Inc.	*Pimephales promelas*	lab, S, SR	juvenile	96 hr	N/A	yes	3 weeks	6

acute fish, continued

LABORATORY	SPECIES	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	HOLDING CAPACITY	TURNAROUND TIME	# RUN AT ONCE
Olver Inc.	*Pimephales promelas*	lab, S, SR	< 90 days	24-96hrs	0	yes	2 weeks	6 to 8
Technical Testing Labs.	*Pimephales promelas*	lab, S, SR	<90 days	24-96 h	N/A	yes	2-3 weeks	1 to 8
UMAB Aquatic Tox. Facility	*Pimephales promelas* *Ictalurus nebulosus* *Ictalurus punctatus* *Salmo gairdneri* *Lepomis macrochirus* *Oryzias latipes* *Carassius auratus* *Fundulus sp.* *Cyprinodon sp.* *Menidia sp.*	lab, S, SR, C for all fish	juvenile and adult for all fish	96 hr for all	0-35 ppt for all fish	yes depends on species	approx. 2 weeks, depending on availability of organisms	varies, depending on size of fish
Versar, Inc., ESM Operations	*Menidia beryllina* *Pimephales promelas* *Cyprinodon variegatus*	lab, S, SR	larva/ juvenile	24-96 hr	0-35 ppt	no	3 wks	3
Virginia Inst. of Marine Science	*Leiostomus xanthurus* *Cyprinodon variegatus* *Fundulus heteroclitus*	lab, S, SR, C	juvenile juvenile & adult	4 days	16-24 ppt	yes	30 days	3

1 "Condition" refers to whether the test is conducted in the laboratory (lab), a mobile lab (mob), or in situ; whether the test is run under static (s), static renewal (sr), or continuous (c) flow conditions; and for some tests, whether it is for water or sediment (sed).

2 In reference to "LIFE STAGE," E, L, and J refer to embryo, larva, and juvenile, respectively.

3 "Holding Capacity" refers whether or not space is available in the laboratory to hold these organisms.

ACUTE INVERTEBRATE BIOASSAYS

LABORATORY	SPECIES	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	HOLDING CAPACITY	TURNAROUND TIME	# RUN AT ONCE
Applied Physics Laboratory of Johns Hopkins University, and the University of Maryland Agricultural Experiment Station	*Palaemonetes sp.*	lab,mob, in situ S, SR, C	juvenile larva &	2-4 days	5-15 ppt	yes	5-6 days	approx. 10
	Mysidopsis bahia	lab, mob, SR, C	post-larva	"	20	"	"	4 to 10
	Mysidopsis bigelowi	"	"	"	5-15 ppt	"	"	"
	Neomysis americana	"	juvenile	"	"	"	"	"
	Callinectes sapidus	"	juvenile	"	"	"	"	"
	Rhithropanopeus harrisii	"	Copepodite	"	"	"	"	"
	Acartia tonsa	lab, mob, SR	embryo, larva,spat	"	10-15 ppt	"	"	"
	Crassostrea virginica	lab,mob,S,SR,C	juvenile	"	5-15 ppt	"	"	"
	Gammarus sp.	"	7 day old	"	"	"	"	"
	Daphnia magna *Daphnia pulex*	Lab,mob,in situ & SR,C for	2-4 days	"	0	"	"	5 to 6
	Ceriodaphnia dubia	both. lab, mob, SR, C	"	"	"	"	"	"
Applied Marine Research Lab. Old Dominion University	*Mysidopsis bahia*	lab, S	3 days old	48 hr	15-25 ppt	yes	20-30 days	15
Biological Monitoring, Inc.	*Daphnia pulex* *Daphnia magna* *Ceriodaphnia*	lab,mob, in situ S, SR, C for all	all	24 -96 hr	0	yes	2 weeks	15
	Mysidopsis sp. *Palaemonetes pugio*	"	"	"	3-33 ppt	"	"	"
Center for Environ. Studies Virginia Tech.	*Daphnia sp.*	lab, S, SR	as prescribed	as prescribed	as prescribed	yes	4-5 days	4 to 6
Coastal Bioanalysts, Inc.	*Mysidopsis bahia*	lab, S, SR	juvenile	48-96 hr	20-34 ppt	yes	1 week	30
	Palaemonetes pugio *Neomysis americana*	"	juvenile	"	"	no	"	"
	Daphnia pulex	"	neonate	48 hr	0	yes	"	25
	Ceriodaphnia dubia	"		"	"	"	"	"
Commonwealth Labs, Inc.	*Ceriodaphnia*	lab, S	not given	48 hr	< 3 ppt	yes	2 days post test	6
	Daphnia	"	"	96 hr	"	"	"	"

acute invertebrate bioassays, continued

LABORATORY	SPECIES	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	HOLDING CAPACITY	TURNAROUND TIME	# RUN AT ONCE
EA Engineering, Science, Tech., Inc.	*Daphnia pulex*	lab, mobile, &	neonate	48 hr	0-2 ppt	yes	30 days	10
	Daphnia magna	in situ and	"	"	"	"	"	"
	Ceriodaphnia dubia	S, SR, and C	"	"	"	"	"	"
	Mysidopsis bahia	for all	larv.- juv.	96 hr	20-30 ppt	"	"	"
	Palaemonetes pugio	"	"	"	"	"	"	"
	Crassostrea virginica	"	spat - juv.	"	5-15 ppt	"	"	"
	Rangia cuneata	"	"	"	5-15 ppt	"	"	"
	Arabacia punctulata	"	"	"	"	"	"	"
	Mytilis edulis	"	"	"	20-30 ppt	"	"	"
Environmental Resources Mgt.	*Daphnia magna* *Daphnia pulex* *Ceriodaphnia dubia*	lab, SR	< 24 hrs	48 hr	0	yes	14 days	7
Environ. Systems Service Ltd.	*Daphnia pulex*	lab, SR	< 24 hrs	96 hr	0	yes	10 days	4
	Daphnia magna	"	"	24 hr	"	"	"	"
Free-Col Labs, Inc.	*Daphnia pulex* *Ceriodaphnia*	lab, SR	neonate	48 hr	0	yes	not reported	12
	Physa sp.	lab, S	< 90 days	"	not reported	"	"	"
Hampton Roads Sanitation Dist.	*Mysidopsis bahia*	lab, S	< 5 days	48 hr	18-22 ppt	yes	6 days	3
	Palaemonetes pugio	"	< 30 days	"	"	"	"	4
Horn Point Environmental Lab. University of Maryland	*Daphnia sp.* *Ceriodaphnia sp.* *Mysidopsis sp.* *Palaemonetes sp.* *Penaeus sp.* *Callinectes sapidus* *Crassostrea virginica*	lab, S, SR	any	any	any	yes	1 week	2
James R. Reed and Associates	*Ceriodaphnia dubia* *Daphnia pulex*	lab, S	neonate	48 hr	0	yes	2 weeks	6
	Mysidopsis bahia	"	juvenile	"	20-24 ppt	"	"	8

acute invertebrate bioassays, continued

LABORATORY	SPECIES	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	HOLDING CAPACITY	TURNAROUND TIME	# RUN AT ONCE
Malcolm Pirnie, Inc.	*Daphnia pulex* *Daphnia magna*	lab, S, SR	neonate	48 hr	0	yes	3 weeks	8
Olver, Inc.	*Daphnia pulex* *Daphnia magna* *Ceriodaphnia sp.*	lab, S, SR	neonate	48 hr	0	yes	2 weeks	15
Technical Testing Laboratories	*Daphnia magna*	lab, S, SR	> 24 hrs	24-96 hr	0	yes	2 - 3 weeks	1 to 8
	Ceriodaphnia dubia	"	"	"	"	"	"	1 to 5
	Daphnia pulex	"	"	"	"	"	"	1 to 3
Versar, Inc.	*Ceriodaphnia, sp.* *Daphnia sp.*	lab, S	< 24 hr old	24-48 hr	0-20 ppt	no	3 weeks	3
	Mysidopsis bahia	"	1-5 days	"	"	"	"	"
Virginia Inst. of Marine Science.	*Mysidopsis bahia*	lab, S, SR, C	neonate	4 days	16-23 ppt	yes	30 days	2
	Palaeomonetes pugio	"	adult	"	"	"	"	3

1 "Condition" refers to whether the test is conducted in the laboratory (lab), a mobile lab (lab), or in situ, and whether the test is run under static (s), static renewal (sr), or continuous (c) flow conditions.

2 *"Holding Capacity" refers to whether or not space is available in the laboratory to hold these organisms.*

BACTERIAL BIOASSAYS

LABORATORY	SPECIES	TEST TYPE	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	TURNAROUND TIME	# RUN AT ONCE
Applied Marine Research Lab. Old Dominion University	*Salmonella typhimiurium* *Vibrio alginolyticus*	Ames Assay respiration	lab, sediment lab, sediment	colonies N/A	2-3 days < 1 day	N/A N/A	4 to 5 days < 1 day	20 - 100 "
EA Engineering, Sci.,Tech.,Inc.	*Photobacterium phosphorium*	Microtox	lab, mobile	N/A	< 1 hour	30 ppt	30 day max.	3 - 10
Free-Col Laboratories, Inc.	Bacteria	Microtox	lab	N/A	5-15 min	N/A	not given	6

BIOCHEMICAL ASSAYS

LABORATORY	SPECIES	TEST TYPE	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	TURNAROUND TIME	# RUN AT ONCE
Applied Marine Research Lab. Old Dominion University	indiginous fish species	blood micro-nuclei assay	field/lab	juv-adult	1 day	N/A	2 days	20 slides per day
Chesapeake Biological Lab. University of Maryland	various fish species	cytochrome P-450 assay	lab, in situ	all	N/A	N/A	1 to 2 weeks	limited by personnel
	bivalves and fish	metal binding protein induct.	lab, in situ	adult	N/A	varies	1 to 2 weeks	"
	molluscs and fish	immunoassay	lab, in situ	adult	N/A	N/A	1 to 2 weeks	"
EA Engineering, Sci, Tech, Inc.	molluscs and fish	bioaccumulation	lab,mob,in situ	juv-adult	2-18 wks	0-30 ppt	30 days	3 per day
UMAB Aquatic Tox. Facility University of Maryland	various fish species	cell culture	lab	N/A	48 hours	0-35 ppt	2 to 3 weeks	5
		explant culture	"	"	as needed	"	"	"
UMBC Biological Sciences University of Maryland	any species	non-enzymatic protein	any	any	5 hours	all	1 day	4 per day
		time to coma	lab	larv-adult	1 hour	all	"	40 at once

CHRONIC FISH BIOASSAYS

LABORATORY	SPECIES	TEST TYPE	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	TURNAROUND TIME	# RUN AT ONCE
Applied Marine Research Lab. Old Dominion University	*Oryzias latipes* (Japanese Medaka)	early life	lab, S	embryo to fry	21 days	0 to 35+ ppt	25 days	250 eggs per day
Applied Physics Laboratory of Johns Hopkins University, and the University of Maryland Agricultural Experiment Station	*Menidia beryllina*	short term	lab, mob, C	< 24 hrs	7 days	0 - 15 ppt	about 10 days	4
		early life	"	eggs	30 days	5 - 15 ppt	about 35 days	"
	Fundulus sp.	short term	"	< 24 hrs	7 days	"	about 10 days	"
		early life	"	eggs	30 days	"	about 35 days	"
		full life cycle	"	"	150 days	"	about 6 months	2
	Cyprinodon variegatus	short term	"	< 24 hrs	7 days	"	about 10 days	4
		early life	"	eggs	30 days	"	about 35 days	"
		full life cycle	"	"	120 days	"	about 6 months	2
	Morone saxatilis	short term	"	larvae	7 days	"	about 10 days	4
	Pimephales promelas	short term	all test conditions	< 24 hours	"	0	about 10 days	4 - 10
		early life	lab, mob, situ, C	embryo	30 days	"	about 35 days	4
		full life cycle	lab, mobile, C	"	6-8 months	"	7 - 9 months	2
	Salmo gairdneri	early life	"	"	60 days	"	about 70 days	4
Biological Monitoring, Inc.	*Pimephales promelas* *Lepomis macrochirus* *Salmo sp.*		all test conditions	all stages	7 - 14 days	0	2 weeks after completion	10
	Cyprinodon variegatus *Menidia beryllina*		"	"	"	3 - 33 ppt	"	"
Center for Environ. Studies] Virginia Tech.	*Pimephales promelas*		lab, S, SR	all stages	as prescribed	as prescribed	7 - 45 days	2 - 3
Coastal Bioanalysts, Inc.	*Cyprinodon variegatus*	survival & growth	lab, SR	< 48 hours	7 days	20 - 34 ppt	2 weeks	14
	Medidia beryllina	"	"	"	"	5 - 34 ppt	"	6
	Fundulus heteroclitus	"	"	"	"	20 - 34 ppt	"	14
	Pimephales promelas	"		"	"	0	"	14
Commonwealth Labs, Inc.	*Pimephales promelas*	larval survival	lab, S	larvae	7 days	< 3 ppt	2 days after completion	6
EA Engineering, Sci., Tech., Inc.	*Pimephales promelas* *Lepomis macrochirus*		all test conditions	< 24 hours	S - 7 days R - 30 days	0	30 days	
	Cyprinodon variegatus		"		"	20 - 30 ppt	"	

chronic fish bioassays, continued

LABORATORY	*SPECIES*	TEST TYPE	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	TURNAROUND TIME	# RUN AT ONCE
Environmental Resources Mgt.	*Pimephales promelas*	survival & growth	lab, SR	< 24 hours	7 days	0	3 weeks	2
Environ. Systems Service, Inc.	*Pimephales promelas*	survival & growth	lab, SR	< 48 hours	7 days	0	15 days	4
Free-Col Laboratories, Inc.	*Pimephales promelas*		lab, SR	< 24 hours	7 days	0	not given	4
Hampton Roads Sanitation Dist.	*Cyprinodon variegatus*		lab, S, SR	< 24 hours	7 days	18 - 22 ppt	17 days	2
Horn Point Environmental Lab. University of Maryland	*Pimephales promelas*	reproduction	lab, S, SR	any	any	any	1 week	2
	Morone saxatilis	repr., growth	"	egg, juv.	"	"	"	"
	Lepomis macrochirus	growth	"	any	"	"	"	"
	Ictalurus punctatus	reproduction	"	"	"	"	"	"
	Fundulus sp. *L. rhomboides* *Cyprinodon variegatus* *Menidia sp.* *Leostomus xanthurus* *Gasterosteus aculeatus*	repr.,growth	"	"	"	"	"	"
James R. Reed and Associates	*Pimephales promelas*	surv.,growth	lab, SR	< 48 hours	7 day	N/A	2 weeks	2
	Cyprinodon variegatus	surv.,growth, and fecundity	"	< 24 hours	"	18 - 22 ppt	"	N/A
Malcolm Pirnie, Inc.	*Pimephales promelas*		lab, SR	24 hours	7 days	N/A	5 weeks	2
Olver, Inc.	*Pimephales promelas*	larval surv. and growth	lab, SR	< 24 hours	7 days	N/A	2 weeks	6 - 8
Technical Testing Labs., Inc.	*Pimephales promelas*		lab, SR	< 24 hours	7 days	N/A	2 - 3 weeks	1 - 3

chronic fish bioassays, continued

LABORATORY	SPECIES	TEST TYPE	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	TURNAROUND TIME	# RUN AT ONCE
UMAB Aquatic Toxic. Facility	*Pimephales promelas* *Ictalurus nebulosus* *Ictalurus punctatus* *Salmo gairdneri* *Lepomis macrochirus* *Oryzias latipes* *Carassius auratus* *Fundulus sp.* *Cyprinodon variegatus* *Menidia sp.*	{All fish for early life stage test}	lab, SR, C	as specified	30 days	0 - 35 ppt	about 2 months	varies
		{All fish for chronic LC50.}	lab, SR, C	as specified	60-90 days	0 - 35 ppt	about 2 months	varies
		{All fish for EC50.}	lab, S, SR, C	as specified	as specified	0 - 35 ppt	about 2 months	varies
Versar, Inc.	*Pimephales promelas*	larv. surviv.	lab, SR	24 hours	7 days	N/A	4 weeks	2
	Cyprinodon variegatus	embryo-larv. survival and teratogenicity	"	embryo	9 days	5 - 32 ppt	"	2
	Menidia beryllina	larv.surviv. and growth	"	7-11 days	7 days	"	"	2
	Morone saxatilis *Fundulus heteroclitus*	in situ	in situ	juvenile	< 30 days	0 - 30 ppt	3 months	1

1 "Condition" refers to whether the test is conducted in the laboratory (lab), a mobile lab (mob), or in situ, and whether the test is run under static (s), static renewal (sr), or continuous (c) flow conditions.

CHRONIC INVERTEBRATE BIOASSAYS

LABORATORY	SPECIES	TEST TYPE	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	TURNAROUND TIME	# RUN AT ONCE
Applied Marine Research Lab.	*Mysidopsis bahia*		lab, S	7 day old	7 days	15 - 25 ppt	30 - 45 days	4
Applied Physics Laboratory of Johns Hopkins University, and the University of Maryland Agricultural Experiment Station	*Mysidopsis bahia*	short term	lab, mob, S, C	7 days old	7 days	5 - 20 ppt	about 10 days	4 - 10
		life cycle	"	post-larvae	"	20 ppt	1 month	"
	Neomysis americana	short term	"	larvae	28 days	5 - 15 ppt	about 10 days	"
	Ceriodaphnia dubia	short term	lab, mob, situ,SR	neonate	7 days	N/A	"	10
		life cycle	lab, mob, SR	neonate	5 - 7 days	"	7 - 9 days	"
	Paratanytarsus parthenogenticus	life cycle	lab, mob, SR, C	egg	about 18 d	"	20 - 22 days	4 - 10
Biological Monitoring, Inc.	*Daphnia pulex* *Daphnia magna* *Ceriodaphnia sp.*		all conditions	all stages	7 - 28 days	N/A	2 weeks	10
	Mysidopsis sp. *Palaemonetes pugio*		"	"	7 - 21 days	3 - 33 ppt	"	"
Center for Environ. Studies Virginia Tech.	*Daphnia sp.* *Ceriodaphnia sp.*	{life cycle & short term}	lab, S, SR	as specified	as specified	N/A	10 days	2 - 3
Coastal Bioanalysts, Inc.	*Mysidopsis bahia*	growth, and fecundity	lab, S, SR	juvenile	7 days	20 - 34 ppt	2 weeks	10
	Ceriodaphnia dubia	surv, reprod	"	neonate	"	N/A	"	16
	Daphnia magna *Daphnia pulex*	surv, reprod and growth	"	"	21 days	"	4 weeks	12
Commonwealth Lab, Inc.	*Ceriodaphnia sp.*	surv, reprod.	lab, S	not given	7 days	N/A	2 days + test	6
EA Engineering, Sci., Tech., Inc.	*Daphnia magna*	short term	lab, mob, SR, C	10 days old	7 days	< 2 ppt	30 day max.	10
		21- day	"	< 24 hours	21 days	"	"	"
	Daphnia pulex	short term	"	10 days old	7 days	"	"	"
		21- day	"	< 24 hours	21 days	"	"	"
	Ceriodaphnia dubia	short term	"	< 2 hours	7 days	"	"	"
	Mysidopsis bahia	28- day	"	7 - 8 days	28 days	< 30 ppt	"	2
Environmental Resources Mgt.	*Ceriodaphnia dubia*	surv, reprod	lab, SR	< 24 hours	7 days	N/A	3 weeks	2
Environ. Systems Service, Ltd.	*Ceriodaphnia dubia*	surv, reprod	lab, SR	<24 hours	7 days	N/A	15 days	4

chronic invertebrate bioassays, continued

LABORATORY	SPECIES	TEST TYPE	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	TURNAROUND TIME	# RUN AT ONCE
Free-Col Laboratories, Inc.	*Ceriodaphnia dubia*		lab, S, SR	< 24 hours	7 days		not reported	4
Hampton Roads Sanitation Dist.	*Mysidopsis bahia*		lab, S , SR	7 - 14 days	7 days	18 - 22 ppt	21 days	2
Horn Point Environmental Lab.	*Daphnia sp.* *Ceriodaphnia sp.*	reproduction	lab, S, SR	any	as prescribed	any	1 week	2
	Mysidopsis sp. *Palaemonetes sp.*	growth, repr.	"	"	"	"	"	"
	Penaeus sp. *Callinectes sapidus* *Crassostrea virginica*	growth	"	"	"	"	"	"
James R. Reed and Associates	*Ceriodaphnia dubia*	surv, reprod	lab, SR	< 24 hours	7 days	N/A	2 weeks	6
	Mysidopsis bahia	surv,growth, & reprod.	"	7 days	"	18 - 22 ppt	"	N/A
Malcolm Pirnie, Inc.	*Ceriodaphnia dubia*		lab, SR	< 24 hours	7 days	N/A	5 weeks	2
Olver, Inc.	*Ceriodaphnia dubia*	surv, reprod	lab, SR	< 24 hours	variable	N/A	4 weeks	5
Technical Testing Laboratories	*Ceriodaphnia dubia* *Daphnia magna*		lab, SR "	> 24 hours "	7 days "	N/A "	2 - 3 weeks "	1 - 3 "
Versar, Inc.	*Ceriodaphnia dubia*	surv, reprod	lab, SR	24 hours	3 broods	N/A	4 weeks	2
	Mysidopsis bahia	surv, growth & fecundity	"	7 days old	7 days	20 - 30 ppt	"	"
Virginia Inst. of Marine Science	*Mysidopsis bahia*		lab, S, SR, C	egg to egg	21 days	16 - 23 ppt	60 days	2
	Crassostrea virginica *Mercenaria mercenaria*	embryo test	lab, S, SR	embryo	2 days	"	30 days	2 - 3

1 "Condition" refers to whether the test is conducted in the laboratory (lab), a mobile lab (mob), or in situ, and whether the test is run under static (s), static renewal (sr), or continuous (c) flow conditions.

PLANT BIOASSAYS

LABORATORY	SPECIES	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	HOLDING CAPACITY	TURNAROUND TIME	# RUN AT ONCE
Applied Physics Laboratory of Johns Hopkins University, and the University of Maryland Agricultural Experiment Station	*Paratanytarsus parthenogenticus*	lab, mob, SR, C	all	2 - 4 days	0	yes	5 - 6 days	4 - 10
	Hydra littoralis	lab,mob,S,SR,C	adult polyp	"	"	"	"	5
	Hexagenia bilinata	lab,mob, S, SR	all	"	"	"	"	10
	Selanastrum capricornutum	lab, mob, S	log phase	4 days	"	"	"	5
	Lemna minor	lab, mob, S, SR	fronds	"	"	"	"	10
Biological Monitoring	Duckweed	all conditions:	all	7 days	0	yes	2 weeks	5
	Selanastrum sp.	lab,mob,S,SR,C	"	96 hours	"	"	"	"
Coastal Bioanalysts, Inc.	*Selanastrum capricornutum*	lab, S	N/A	96 hours	0	yes	1 week	3 - 6
	Skeletonema costatum	"	"	"	20 - 34 ppt	"	"	"
	Champia parvula	"	male/female branches	7 days	30 ppt	no	2 weeks	14
EA Engineering, Sci., Tech., Inc.	*Selanastrum capricornutum*	lab, S	10 day old culture	96 hour to 14 days	0 - 2 ppt	yes	30 days	3
	Skeletonema costatum	"			20 - 30 ppt	"	"	"
Environ. Resources Management	*Selanastrum capricornutum*	lab, S	4 to 7 days	96 hour	0	no	14 days	2
Environ. Systems Service, Ltd.	*Selanastrum capricornutum*	lab, SR	not given	7 day	N/A	yes	15 days	4
Horn Point Environmental Lab.	Various	lab, S	log phase	andy	any	no	1 week	2
James R. Reed and Associates	*Selanastrum capricornutum*	lab, S	log phase	96 hours	N/A	yes	2 weeks	N/A
Malcolm Pirnie, Inc.	various algae and duckweed	lab, S	N/A	2-14 days	varies	yes	4 weeks	2 - 5
Olver, Inc.	*Selanastrum capricornutum*	lab, S	4 - 7 days	96 hours	N/A	yes	4 weeks	5

plant bioassays, continued

LABORATORY	SPECIES	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	HOLDING CAPACITY	TURNAROUND TIME	# RUN AT ONCE
Patuxent Wildlife Research Center	*Potamogeton sp.* (autotrophic system and microcosm)	lab, S, SR	vegetative	5 weeks	variable	yes	5 weeks	2 - 3
	(photosynthesis test)	lab, S	"	3 hours	"	yes	2 days	2 per day
	(floating pods)	in situ	"	5 weeks	"	"	6 weeks	6

1 "Condition" refers to whether the test is conducted in the laboratory (lab), a mobile lab (mob), or in situ, and whether the test is run under static (s), static renewal (sr), or continuous (c) flow conditions.

2 "Holding Capacity" refers to whether or not space is available in the laboratory to hold these organisms.

SEDIMENT BIOASSAYS

LABORATORY	SPECIES	CONDITIONS	LIFE STAGE	TEST LENGTH	SALINITY	HOLDING CAPACITY	TURNAROUND TIME	# RUN AT ONCE
Applied Marine Research Lab. Old Dominion University	*Palaemonetes pugio*	lab, sediment,S	adult	10 days	15 - 25 ppt	yes	45 days	6
	Mytilus edulus	"	"	"	"	"	"	"
	Cyprinodon variegatus	"	< 90 days	"	"	"	"	"
	Mysidopsis bahia	suspended solid	< 5 days	48 hours	"	"	"	10
	Cyprinodon variegatus	"	< 90 days	96 hours	"	"	"	8
	Lepidactylus dytiscus	lab, sed, S	juv, adults	10 days	15 - 30 ppt	"	"	10
EA Engineering, Sci., Tech., Inc.	various	lab, mob, in situ, S, SR,C	early life to juvenile	96 hours to 30 days	0 - 30 ppt	yes	30 day max.	3 - 10
Versar, Inc.	*Chironomus tentatus*	lab, sediment,S	2nd instar	< 25 days	N/A	yes	4 weeks	2
	Hyallela azteca	"	juvenile	< 10 days	0 - 30 ppt	"	4 weeks	"

1 "Condition" refers to whether the test is conducted in the laboratory (lab), a mobile lab (mob), or in situ, and whether the test is run under static (s), static renewal (sr), or continuous (c) flow conditions. It is also specified whether the test involves the use of sediment or suspended solid.

2 "Holding Capacity" refers whether or not space is available in the laboratory to hold these organisms.