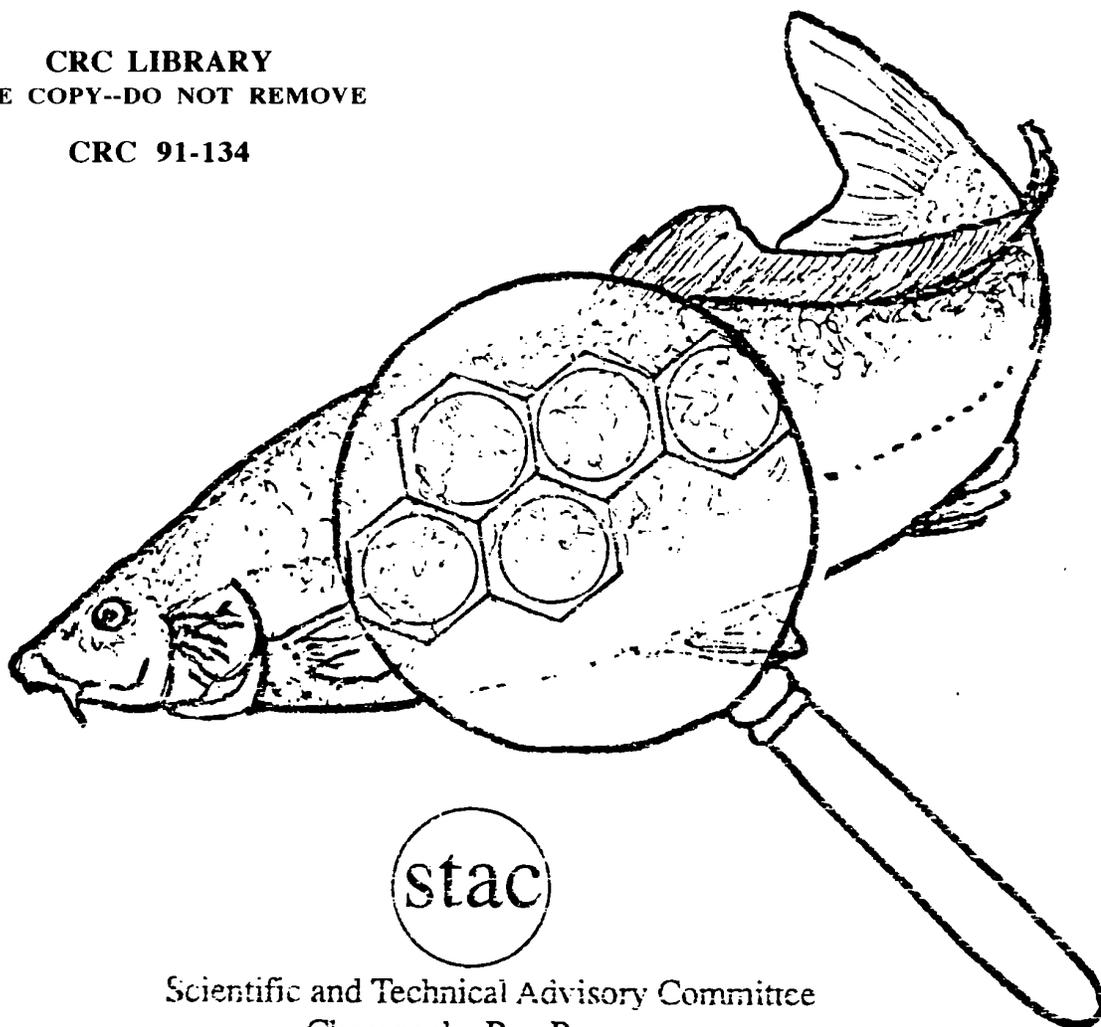


Chesapeake Toxicological Workshop Series

Executive Summary

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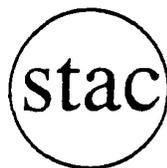
Scientific and Technical Advisory Committee
Chesapeake Bay Program

Chesapeake Toxicological Workshop Series

Executive Summary

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July 1991



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Table of Contents

FOREWORD	1
BIOMARKER WORKSHOP	
Synopsis	3
Participants	7
WHOLE ORGANISM WORKSHOP	
Synopsis	9
Participants	13
FATE AND TRANSPORT WORKSHOP	
Synopsis	15
Participants	21
RISK ASSESSMENT WORKSHOP	
Synopsis	23
Participants	30
Appendix A - AMBIENT TOXICITY WORKSHOP	
Suborganismal Session Report	31

FOREWORD

The following reports are the result of a series of workshops held in 1990-1991. The United States Environmental Protection Agency (EPA) and the National Oceanographic and Atmospheric Administration (NOAA) requested that the Chesapeake Bay Program's Scientific and Technical Advisory Committee (STAC), with assistance from the Chesapeake Research Consortium (CRC), organize the workshops to deal with key contaminant issues as related to Chesapeake Bay system processes and living resources.

Federal and state agencies recognize the need to accelerate our contaminant research and management activities in the overall Bay restoration effort. Accordingly, increasing levels of federal and state funding will continue to develop "Requests for Proposals" for appropriate research projects in toxic contaminants. These workshop executive summaries are meant to provide federal and state agencies with the most current and objective recommendations from the scientific community as to the most appropriate and useful guidelines for developing the "Request for Proposals" documents.

The workshops addressed major categories of toxicological research, including suborganismal toxicity testing, whole organism toxicity, contaminant fate and transport, and ecological risk assessment. A few recommendations were common to all the workshops:

- The need to integrate related research programs in diverse fields such as physics, chemistry, physiology, toxicology and ecology.
- The importance of determining the relationship between suborganismal indicators of exposure and actual adverse effects of toxic chemicals on populations.
- The need to systematically acquire baseline ecological data to assist in the interpretation of toxic effects.

For the last several years, the Chesapeake Bay Program has sharpened the focus on the issue of toxic contamination. In 1989, STAC and CRC held an "Ambient Toxicity Assessment Workshop" and subsequently published recommendations for ambient toxicity studies in the Chesapeake Bay. The 1990-1991 workshops attempt to fill information gaps and provide an updated critical assessment of where the Bay Program's toxics/toxicology research should be directed. The recommendations contained in the following pages in many ways represent the cutting edge of scientific thought in estuarine/ aquatic toxicology. They are the consensus findings of active researchers from throughout the Bay region in addition to investigators from leading research institutions in North America. I believe the information herein will be useful in directing research programs, and that the questions raised through this process will provide a basis for further workshops on related issues.

Joseph A. Mihursky,
STAC Chair and CRC Director

BIOMARKER WORKSHOP SYNOPSIS
Jay Gooch and Robert Huggett (co-chairmen)
September, 1990

Introduction

For many years, aquatic scientists have suggested using biochemical and physiological measurements to detect sublethal effects of chemicals on aquatic animals. The ability to detect fundamental molecular changes that precede apparent adverse effects will increase our understanding of how contaminants affect resident aquatic organisms and ultimately will help us protect aquatic populations. The suborganismal response to contaminants is often collectively referred to as "biomarkers".

Recently, a series of regional, national and international workshops discussed the use of biomarker techniques in a pollution monitoring context (Chesapeake Bay Ambient Toxicity Assessment Workshop Report, 1989 see Appendix A). The biomarkers discussed at the STAC workshop in November focused on the contaminants known to be present in Chesapeake Bay waters (Helz and Huggett, 1987); the information provided below reflects the collective wisdom and expertise of the workshop participants.

While there is often discussion over whether biomarkers are measures of exposure or measures of effect, it is perhaps best to describe biomarkers as *effects which can be used as measures of exposure*. Clearly, the negative impact of the chemical will depend on the magnitude of the biomarker response as well as the type of process that is affected. More research is necessary to fully understand the nature of these linkages.

As with any bioassay, there are limits to the amount of extrapolation that can be made from the results, however, biomarker responses can be used as sensitive, sublethal markers of biologically significant exposure. These responses integrate information about other important factors including bioavailability (if compounds were not sufficiently bioavailable, no response would be seen) and spatial and temporal variability (many of these responses have the potential to integrate over space and time scales different from those over which the chemical exposure is operating).

Background

Biomarkers can be lumped into two broad categories; 1) those that indicate exposure to specific contaminants, and 2) those which simply indicate a general stress condition. Those in which organisms are examined for a contaminant-specific response can then be related back to exposure to specific chemicals or classes of chemicals. This type of

mechanistic approach is possible when a defined cause and effect linkage exists. Examples of such responses include the induction of monooxygenase enzymes (proteins responsive to planar aromatic pollutants in fishes), metallothioneins (proteins responsive to several types of trace metals), and acetylcholinesterase (an enzyme which is specifically inhibited by organophosphate and carbamate insecticides). Many years of study have shown that these enzymes and proteins respond in very specific and predictable ways following exposure of the host organism to specific classes of chemicals. Based on these studies, it is possible to ask focused questions regarding the exposure of indigenous organisms to specific classes of chemicals by measuring biochemical responses.

The other broad category of biomarkers includes many general endpoints/effects which have been measured in organisms exposed to a wide variety of toxicants. These endpoints include, but are not limited to, measures of various biochemical constituents, such as hormones, free amino acids, adenylates (ATP, etc.), energy reserves (lipids), and serum enzymes. Changes in systems such as the immune and genetic systems also can be included in this category of general responses.

Histopathological analysis is another technique which can be classified as a general biomarker. For example, many water quality researchers use lesions in feral organisms as an environmental indicator and the role of this technique in environmental monitoring is an important one.

While changes in such nonspecific endpoints can be related to chemical exposure, they cannot be used to implicate or discern chemical exposure with as much certainty as the more specific biomarkers. The lack of specificity does not preclude their use per se. In many cases, these general indicators of exposure may be most valuable when used along with other more specific biomarkers and/or actual measures of chemical body burden or exposure. These more general measures may reflect the combined effects on multiple biochemical pathways, in the case of complex exposures, as well as the integration of specific effects of single toxicants on multiple tissues within an organism. At this stage, it is clear that a constellation of biological parameters may be necessary in order to adequately define the consequences of chemical exposure in indigenous organisms. More research is required to evaluate appropriate parameters and to define the best context for their use.

Research Strategy

In order to address the following research needs, a combination of laboratory and field studies should be used. Since many biomarker tests are specialized, the expertise to conduct the tests may exist only in discrete laboratories. For this reason, we need to conduct collaborative studies using a multi-investigator consortium. In doing so, samples should be shared where possible so that various endpoints from tissue subsamples can be tested and compared.

While numerous biomarker techniques have been developed, additional information is needed to make these tools more useful for pollution assessment in Chesapeake Bay. Through laboratory studies, we need to investigate the specific properties of biomarker endpoints in species resident in the Chesapeake System. Also, field investigations should be conducted in a series of Chesapeake Bay locations known to be polluted to varying degrees. Species and tests are recommended for biomarker research based on their distribution in Chesapeake Bay and the biomarker expertise in the Bay region. Specific suggestions are also given for locations at which biomarker investigations should be conducted. These include "hot spots" and areas with well-known pollution gradients. All studies should be conducted with the appropriate chemical measurements.

Research Needs

The Biomarker Workshop Panel has identified the following research needs. The needs are not necessarily listed in order of priority.

1. Baseline Information on Resident Chesapeake Bay Species

We need to determine basic information about the sensitivity and range of the biomarker responses in bioassay species and indigenous organisms in Chesapeake Bay. Many of the biochemical systems that are targets of chemical contaminants appear to be comparable in different types of organisms so some direct extrapolation is already possible.

- ***Establish baseline information on Resident Chesapeake Bay Species***
We need to establish baseline information on the sensitivity and range of the biomarker responses in bioassay species, and especially indigenous Chesapeake Bay organisms. These studies should be carried out using model compounds (reference toxicants) known to exhibit a particular mode-of-action.
- ***Determine breadth, sensitivity and robustness of biomarker responses***
We should design field experiments to detect the possible breadth of biomarker responses in the living resources of the Bay, and also to allow comparison of endpoints for sensitivity and robustness. We should complement these experimental data with the appropriate chemical information (tissue residues, sediment concentrations, etc.). Other commonly used metrics such as benthic infaunal surveys would also complement the experimental data.
- ***Investigate biomarker responses in invertebrates***
The majority of the research on biochemical effects of contaminants in aquatic organisms has been done on fish. We understand much less about invertebrate systems, though several bivalve species (particularly marine) have received considerable attention. We need to develop biomarker techniques to measure responses in aquatic invertebrates.

2. Relating Biomarkers to Whole Organism Responses

We need to clarify the roles of whole organism and biomarker approaches in pollution assessment studies designed to detect contaminant impacts in ecosystems. There are gaps in our understanding about how to relate endpoints measured at different levels of complexity. In the whole organism approach researchers use bioassays to determine the habitability of the water and sediments. In this way, they can answer the question: "Can organisms live, grow and reproduce in samples of representative parts of the real world?" In the biomarker approach scientists sample indigenous organisms and analyze them, or their tissues, for changes which are thought to be related to chemical contaminants. We now recognize that both approaches have merit and should be viewed as complementary parts of the information needed to detect and understand contaminant impacts in ecosystems. For these reasons, we need a better understanding of how to relate biomarker responses to adverse effects on individuals and populations.

- ***Clarify roles and relationships***
There is a need to clarify the relationship between measures made at different levels of organization. To this end, the group recognized a continuing need for research into understanding the relationship between biomarker endpoints and more severe whole animal responses (mortality, depressed fecundity, depressed growth, etc.) measured in toxicity bioassays.

- **Develop biomarkers for use in standard bioassay organisms**
One way to clarify roles and relationships is to incorporate biomarker measurements in bioassays. However, more research is required to determine which biomarker endpoints are applicable / or amenable with standard toxicity bioassay protocols and how bioassays can be modified to incorporate biomarker endpoints.
- **Determine the relative sensitivity of endpoints**
To compare the sensitivity of biomarker and whole organism endpoints we should conduct lab experiments with model toxicants and toxicants which have been reported at elevated concentrations in the Bay (or which are on the Toxics of Concern list). This approach would allow comparison of the relative sensitivities of various endpoints to different concentrations of contaminants and also allow the consideration of multiple toxicant interactions.

Suggested Species and Locations for Biomarker Research

Species :

Fish: Mummichog (*Fundulus heteroclitus*), spot (*Leostomus xanthurus*) white perch (*Morone americana*), striped bass (*Morone saxatilis*) and channel catfish (*Ictalurus punctatus*); Oyster (*Crassostrea virginica*);

Birds: Black-Crowned Night Heron.

Biomarkers for immediate field and lab studies :

Monooxygenase Enzymes (Cytochrome P-450), metallothioneins, DNA adducts and other measures of genotoxicity, immune function, histopathology, serum chemistry, biliary metabolite profiles, heat shock proteins, enzymes related to redox balance.

Biomarkers which require further basic research before application to field studies :

Heme pathway enzymes, porphyrin profiling, measures of endocrine status.

Locations considered appropriate for initial field investigations:

Elizabeth River, Baltimore Harbor, Potomac River, Patuxent River. Where possible and where consistent with the goals of the study, every effort should be made to utilize information being generated by other studies in the Bay (e.g. the ambient toxicity pilot project and sediment toxicity surveys.)

**Biomarker Workshop Participant List
6-7 September 1990**

Name	Affiliation
Bob Huggett	Virginia Inst. of Marine Science
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	Co-Chairman
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Bruce Fowler	University of Maryland at Baltimore
Robert Hale	Virginia Inst. of Marine Science
William Hargis, Jr.	Virginia Inst. of Marine Science
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Eric B. May	Maryland DNR/OCBL
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 Recorders	
Kevin Kiley	
Jackie Savitz	

WHOLE ORGANISM WORKSHOP SYNOPSIS
David Wright and James Pratt (co-chairmen)
November, 1990

Background

Ever since the passage of the Toxics Substances Control Act (TSCA) in 1976, development and application of acute toxicity tests (ie. using mortality as an endpoint) has been driven by regulatory needs. Although these tests provided acute toxicity data, they did not address questions related to specific effects at the chemical level, or effects that could occur under ambient conditions. Furthermore, most ambient measures of chemical concentrations are taken in the sediments while water concentration data are few and far between.

These considerations have contributed to a general lack of useful information on the presence and effects of toxic contaminants in the Bay. The Whole Organism Workshop panel identified a series of four major research needs which should be addressed through systematic research programs in the near-term. Specific recommendations or research needs are also specified.

Research Strategy

Research aimed at determining levels of toxic contaminants in the Bay should address the spatial scale of toxicity. This spatial dimension of potential toxic impacts may depend on transport kinetics of given contaminants, natural seasonal trends, and the distribution of potentially affected organisms. These factors should be considered in designing research and monitoring programs.

The effects of a contaminant can be assessed at a number of different levels ranging from the cellular or molecular level (biomarkers) to the community (multispecies) level. In order to maximize the utility of toxicological results from the management community, it is important that toxic effects are quantified at the highest possible level of organization, i.e. the population or the community.

Biomarkers, or suborganismal effects such as the induction of enzyme pathways, are advantageous endpoints due to the speed of both the toxic response and its measurement. However, the response itself does not necessarily represent an adverse effect on the individual organism. We need to determine which biomarker endpoints clearly predict adverse effects on processes such as reproduction, growth, behavior, etc. in individual organisms, and we need to know what conditions elicit these effects.

Once adverse effects on whole organisms are observed, there are still many factors at work which may compensate for an adverse effect on individuals, thus causing no overall effect on the recruitment of progeny into the population. Such factors include relaxed predation or competition due to either the polluted conditions or to the low initial numbers of individuals. Hence, effects on individuals do not necessarily imply effects at the population level. Therefore, we need to consider the toxicological effects on individuals in the context of these other factors in order to quantify effects at the population or community level for management purposes. Finally, since the sediments are the principal sinks for toxic chemicals, we need to investigate the effects of contaminated sediment on organisms.

We recommend that toxicological research be conducted in a concerted fashion in order to relate the various levels of organization. This will help us to assess effects at the highest practical level of organization while still maintaining a cause-and-effect relationship. This concerted effort should include toxicity testing at both suborganismal and whole organism levels as well as coordinated sediment and water chemical measurements.

Research Needs

The Whole Organism Workshop Panel has identified the following research needs. The needs are not necessarily listed in order of priority.

1. The Spatial Scale of Toxicity

Regulatory toxicity testing is insufficient for dealing with the management needs of the Chesapeake Bay. This inadequacy stems from problems of chemical complexity such as effects of mixtures of chemicals, and biological complexity including interspecific interactions that may either exaggerate or diminish the effects. Since it is not feasible to mount a biological monitoring effort of sufficient scale to cover all potentially contaminated sites, it will be necessary to concentrate on areas with identifiable problems. However, background data demonstrating toxic conditions at ambient levels are limited in spatial scope. There was a consensus among the group that existing problems should first be demonstrated and then trends could be monitored. The Biomarker Workshop also discussed the need for targeting problem areas in research programs.

- ***Develop screening tests***
We need to develop screening tests which are both sufficiently sensitive to detect problem areas and can identify classes of responsible chemicals.
- ***Co-ordinate sediment and water chemistry***
Coordinated chemical measurements between sediment and water are essential for correlating toxicity data with elevated levels of contaminants. Recommendations from the Fate and Transport Panel also address this issue.
- ***Focus on Chesapeake Bay animals that spawn in the Bay***
To better define differential spatial impacts, research should focus on important spawning animals such as the striped bass. This was also addressed by the Biomarkers Workshop.
- ***Characterize time trends***
To identify long term trends and address the effects of seasonality, we need to re-test sites over time. Moreover, representative sites need to be carefully chosen.

- ***Calibrate effluent endpoints against ambient endpoints***
To make effluent testing data more useful for predicting ambient effects, we need to better calibrate these two testing endpoints. For this reason, effluent testing should include more sub-acute assays than used presently.
- ***Implement synoptic chemical monitoring over long time periods***
Synoptic chemical monitoring should be conducted. EPA's Environmental Monitoring and Assessment Program (EMAP) represents a good example of this type of approach.

2. Relating Organism-Level Effects to Population-Level Effects

Once the effects of contaminants on individuals is understood, it is necessary to predict the effects on natural populations for management purposes. Physiological compensatory mechanisms and density-dependent ecological effects potentially may counteract the deleterious effects of contaminants. These types of phenomena have not been adequately addressed in the past but we need a better understanding of them in order to adequately assess toxicant effects.

- ***Investigate population dynamics***
We need a better understanding of long-term, intra-and inter-specific population processes such as predation and competition. These population data will help determine whether adverse effects on individuals translate into adverse effects on whole populations.
- ***Consider mesocosm studies***
Surrogate laboratory environments, or mesocosms, may be a useful mechanism for understanding population processes. Experimental mesocosms, may be a useful mechanism for understanding population processes. Experimental mesocosms should be considered as a viable option for investigating toxic chemical effects at the population and community levels. The EPA supported SAV research successfully used this approach in the Bay.

3. Relating Biomarker Effects to Whole Organism-Level Effects

Biomarkers can provide relatively inexpensive, rapid alternatives to very comprehensive chemical monitoring programs. However, the observance of a biochemical indicator of exposure does not necessarily indicate adverse effects on the individual, therefore no adverse effects on the population would be expected. For example, a strong immune system response to a toxic chemical may, in itself, be a healthy response and could have considerable survival value. Many biomarker effects may reflect survival responses as such. The Biomarkers Workshop also recommended that this relationship be addressed.

- ***Relate biomarker indicators to whole organism tests***
Researchers need to correlate sub-organism or "biomarker" indicators of toxic stress to end-points derived from whole organism tests. In order to correlate and assess the relative sensitivities of these two approaches, researchers need to share samples and conduct integrated water column, sediment, and sub-organismal ambient toxicity tests. Some ongoing studies are addressing this question, but we need more of these studies to accurately assess the relationships between biomarkers and individual level effects.

FATE AND TRANSPORT WORKSHOP REPORT
James Sanders and Gregory Cutter (co-chairmen)
November, 1990

Introduction

In late November 1990, a workshop was convened to discuss the research needs in the field of transport of contaminants through coastal zones, in particular, the Chesapeake Bay. The workshop was sponsored by the Chesapeake Research Consortium and the Scientific and Technical Advisory Committee of the EPA Chesapeake Bay Program and was attended by 19 scientists (See attached list). The goal of the workshop was to develop a concise document describing the issues at hand, identifying important gaps in our understanding, and developing a research strategy that could be used to meld the geochemical, physical, and biological aspects of contaminant transport and fate together into an integrated research program. This document is the result of this workshop.

The focus of this document is primarily the Chesapeake Bay; however, most of the issues identified are applicable to many coastal systems. It is our hope that scientists and managers will find this document of value for other systems.

Background

We have adopted the term "contaminant" to signify any potentially toxic substance that is present in elevated concentration. This convention allows us both exclude and include naturally occurring elements which can be necessary nutrients at low concentrations but toxic at higher concentrations.

Potentially toxic elements and compounds are introduced into the coastal environment through natural and anthropogenic pathways. These contaminants can be retained by the system, they can be chemically altered, or they can pass through unaltered. This processing in the coastal environment is controlled by a complex interaction of chemical, biological, and physical factors. However, our understanding of these interactions is insufficient to predict the fate, distributions, and effects of the majority of contaminants in natural waters. In addition, we are not yet able to establish quantitative linkages between contaminant inputs and the level of exposure borne by organisms. In view of the number and types of contaminants, both organic and inorganic in nature, and the extremely dynamic physical nature of the coastal zone, a comprehensive research program that examines the transport and fate of contaminants in Chesapeake Bay should be undertaken.

In coastal ecosystems such as Chesapeake Bay, there are areas within the system that have been clearly impacted by anthropogenic inputs and other areas that appear to be relatively unaffected, even though contaminants are present in elevated concentrations. The inputs of a wide variety of contaminants continues. We need to better understand how systems process and react to elevated contaminant levels. We must also more fully understand the complex structure of estuarine ecosystems in general in order to identify current problems in the Chesapeake Bay, and to protect resources in future years. Through such research we will build a predictive capability and the ability to respond to future problems that the Chesapeake Bay may face. In addition, such understanding will allow the assessment of impacted areas and will provide the necessary expertise to deal with remediation issues. Therefore, the goals of a research program to address issues in contaminant processing and transport are:

1. To understanding the physical, geochemical, and biological factors that control the distribution, movement, and reactivity of contaminants within coastal systems like the Chesapeake Bay; and
2. Determine how contaminants interact with biota, move through the food web, and impact community and ecosystem structure.

Research Strategy

The transport of particulate and dissolved contaminants in coastal ecosystems like the Chesapeake Bay is controlled largely by physical processes. Tidal currents, estuarine gravitational circulations, and other persistent currents are probably the dominant influence on the transport of dissolved substances. Episodic events, such as storms, may be of equal or greater importance for the transport of particulate material. Transformation and final disposition of contaminants within this physical context, however, is largely controlled by biological and chemical processes. All of these processes interact, and all are characterized by large spatial and temporal heterogeneities in shallow, nearshore waters.

Research strategies for addressing contaminant transport and fate therefore must be inherently interdisciplinary and must consider spatial and temporal variability. Sampling and experiments should be designed and conducted in a coordinated fashion. We also recommend continued and increased use of moored instrumentation whenever possible. Such arrays can help to provide the temporal resolution that ship-based sampling cannot, and allow the sampling of brief episodic events. Without careful design and integration, inadequate data are likely to result.

Another important aspect of contaminant research is the continuing development of new methodologies, particularly for the determination of contaminant levels and chemical forms at ultra trace concentrations. Both monitoring and research efforts must strive to develop better analytical methods and to ensure that they are utilized within all aspects of the program.

Organisms also affect the movement and impact of contaminants within systems. They supply both dissolved and particulate organic carbon compounds to the system which are capable of affecting complexation and partitioning reactions. They also act as physical filters, leading to concentration within the organism or retention in restricted habitats. In addition, organisms functions as biological reactors, repartitioning, transforming, and decoupling/recoupling contaminants as they pass through the gut. Biological processes, including microbial ones, therefore, must be considered in experimental and field design.

In addition to field and experimental research, a modeling component is necessary. Models for the behavior of contaminants in an estuary should be process-oriented, and should therefore accurately represent biogeochemical mechanisms and rates. Any model for contaminants must be developed concurrently with field and laboratory research in order to allow "feedback" between those engaged in modeling and those conducting research. Thus, appropriate models with relatively simple computer codes will be developed, and should be capable of incorporation into larger, system-wide models in the future.

Research Needs

Within the general problem stated above, there are a number of issues that must be addressed. These are described below, and assorted into two general topics, Inputs and Transport, and Biogeochemical Processing. Many of these issues can and should be addressed concurrently, as part of a multidisciplinary effort. Some of these issues are relatively straight-forward, and can be examined in a short time period with existing methodology. Others will require a longer period of study and the development of new approaches. We note with each issue its relative standing.

1. Inputs and Transport

- ***Improve quality of monitoring data (for various terms)***

Contaminants can be introduced to estuarine systems like the Chesapeake Bay through riverine input, atmospheric deposition, coastal seawater inflow (i.e., estuarine circulation), point sources, and non-point sources (e.g., runoff, groundwater). Existing monitoring programs are examining contaminants in riverine, atmospheric, and point-source inputs, and it is assumed that these efforts will continue for the foreseeable future. However, it is imperative that quality data for inputs be obtained. In particular, detection limits for contaminants must be sufficiently low enough to allow their input fluxes to be calculated. Interactions (including intercalibrations) between monitoring agencies and research laboratories using state-of-the-art analytical techniques should be increased to improve the usefulness of monitoring data. These needs are similar to those considerations and developments made for the nutrient tracking in the Bay system.

Two amendments to existing monitoring efforts should be initiated. First, to calculate dry depositional fluxes from the atmosphere, it will be necessary to supplement aerosol measurements with estimates of dry depositional velocities in the marine boundary layer (i.e., over the water) using state-of-the-art methods such as cascade impactor technology. Second, quantification of contaminants from non-point sources, especially runoff and groundwater, is needed. The use of "unique tracers" (e.g., radionuclides, natural organic compounds) might assist in apportioning the relative contributions of each source. Tracers might be particularly useful in quantifying groundwater inputs. These issues can be addressed immediately.

- ***Conduct physical studies concurrently with biogeochemical studies***

Once a contaminant is delivered to an estuary, its mass transport is largely controlled by physical processes, including circulation and mixing within the water column, and resuspension and transport of deposited particles. These processes are highly variable in both time and space. Physical flow, mixing, and transport studies should be carried out concurrently with process-oriented biogeochemical investigations.

- **Determine temporal and spatial variability of mass transfer coefficients across air sea interface in the coastal environment**
 With respect to transfer across the air/sea interface, the temporal and spatial variability of mass transfer coefficients for gases (or "transfer velocities") need to be determined in the estuarine environment. While these coefficients have been determined in the open ocean, the unique orographic, meteorological, and geochemical (e.g. salinity, surface microlayer) properties of the coastal environment indicate that they should be measured here. These measurements can be done immediately with existing methods.
- **Develop methods to sample the air/sea interface**
 The surface microlayer is known to be enriched in many organic and inorganic compounds, as well as micro-organisms, and likely plays a role in sea/air exchange and biogeochemical transformations of contaminants. However, existing methods for sampling and defining this interface are inadequate, and further methods development should occur before considerable research efforts are focused on the microlayer. Therefore, this issue can only be addressed in the long term, as methods and technologies are developed.
- **Determine the role of floc layers in contaminant transfer**
 At the sediment/water interface a "floc" or "fluff" layer of extremely high porosity sediment is commonly found in estuarine systems. Since these highly mobile layers, sometimes called fluidized muds, have high surface areas, organic content, and microbial activity, they may be crucial in the transfer of contaminants across the sediment/water interface. Moreover, particles in floc layers are easily resuspended and may be repeatedly cycled between suspension and deposition before permanent burial. Accordingly, studies of the physical, chemical, and biotic characteristics of floc layers and their role in the transfer of contaminants across the sediment/water interface are needed. These studies should be conducted under varying physical (e.g. actively mixed, quiescent), biological (e.g., bioturbated, undisturbed), and geochemical (e.g., oxic, anoxic) conditions. Because methods largely exist to address this issue, studies could be done immediately.
- **Determine the spatial and temporal variability of sediment resuspension**
 Sediment resuspension affects the vertical flux of fine particulate material across the benthic interface and the net transport of particles from their sources to their ultimate sinks. Resuspension is controlled by a constantly changing balance between physical forcing and sediment stability, and is an event-dominated process. Thus, the spatial and temporal variability of resuspension must be determined in an estuary in order to assess its importance to contaminant transport. This task could be performed with existing methods.
- **Investigate particle dynamics on relevant temporal and spatial scales.**
 Particles in the water column, whether resuspended sediments, biogenic detritus, or clay minerals, transport contaminants and act as sites for their adsorption/desorption. Thus, the processes that control particle dynamics, including episodic events, and the detailed chemical composition of estuarine particulate matter need to be examined on relevant temporal and spatial scales. This is an issue that will require longer-term study.

- ***Assess the role of biota in contaminant transport***
We need to understand the role of biota in the transport of contaminants. In general, organisms act as a concentrating mechanism for contaminants, both through incorporation in tissues and through "biopackaging" via fecal pellets, pseudofeces, etc. This process may act as a mechanism to enhance microbial reactions and to stimulate the induction of mechanisms to facilitate degradation. The relative importance of the transport of contaminants through predator/prey interactions is not well known for many contaminants within an estuary. Furthermore, information available for freshwater and marine systems may not be applicable. Fecal pellets can be an efficient transport mechanism, but we do not know how important it is for Chesapeake Bay, where the generally shallow depth leads to frequent resuspension of bottom sediments. The direct deposition of phytoplankton blooms may also be an important transport mechanism. Again, its relative importance is unknown. This issue can be studied immediately; however, continuing development of new approaches and methods will be required for a complete understanding.

2. Biogeochemical Processing

- ***Perform mechanistic studies of biodegradation and transformation***
The fate and transport of contaminants may be modified by biologically-mediated reactions, the rates and extent of which are currently ill-defined for most of the contaminants which impact Chesapeake Bay. Biotransformation reactions can serve as both a detoxification and activation mechanism. In this regard, enzymatic oxidation (i.e. epoxides) within organisms may alter the toxicity of contaminants. Microbially-mediated reactions also have the potential of transforming parent contaminants. If more soluble products are formed, their transport characteristics would be modified. Considering these processes, mechanistic studies examining the biotic degradation / transformation of contaminants in sediments and the water column should be performed. These studies need to examine this processing as a function of contaminant levels, sediment composition (e.g. organic constituents) and morphology, microbial populations, and redox state. The abiotic mechanisms (e.g., photolysis, hydrolysis, redox reactions) transforming or degrading contaminants must also be studied in a similar fashion. This research can be initiated immediately using existing knowledge; however, as methods develop for assessing biochemical components within organisms and cells, further advances will be made.
- ***Develop methods to investigate temporal and spatial variability of chemical speciation***
In the water column the reactivity and bioavailability of dissolved contaminants are a function of their chemical speciation. Therefore, the geochemical (e.g., salinity, pH, redox state) controls of this speciation must be elucidated. Moreover, the temporal and spatial variability of the speciation of organic and inorganic contaminants should be examined. For the metal ions, speciation studies can utilize techniques such as electrochemical measurements of complexation capacity. However, for organic contaminants, suitable methods need to be further developed. A linkage between metals and organic contaminants could include studies of the abundance and types of complexing ligands. This research will require continuing development of technology and methodologies appropriate for identifying discrete ligands and complexes, and therefore will require a sustained, long-term effort.

- ***Quantify the effects of biota on the bioavailability of contaminants***
 Because biota provide important complexing ligands and are themselves organic-rich particles, it will be important to quantify the alteration in bioavailability due to various classes of complexing ligands and particles to various types of organisms. Sorption to particles may alter exposure to filter feeders. It will also be important to understand the dynamics and lability of complexation and partitioning reactions. Some of this issue can be addressed immediately, other aspects will require new methods as above.
- ***Assess the effects of communities on contaminant transport and transformation***
 The organization of individuals and populations into communities will also affect contaminant transport and transformation. To date, studies usually have dealt with single species and have missed the integrative effects that a community can have on a contaminant. The response of a community is not just a summation of its parts; different trophic levels and microhabitats can combine to yield an unexpected result. An estuarine system like the Chesapeake Bay is composed of many different kinds of communities and biological zones; these may interact with contaminants in different ways. Of particular importance (and often overlooked) is the role of fringing communities: wetland, shallow subtidal plant communities, and benthic and planktonic communities within the tributaries which may be subjected to higher than average contaminant inputs. This research can be initiated using existing knowledge.
- ***Determine the effects of benthic processes on contaminant fate and transport***
 We need to better understand how benthic processes affect transport and fate of contaminants. Benthic organisms with different lifestyle activities (filter feeders, deposit feeders, conveyor belt feeders, etc.) will have different effects on physical and chemical processes in sediments through differing degrees of sediment movement and impacts on redox zones. How do the major feeding pathways (filter feeders, deposit feeders, water column grazers) affect contaminant transport and if the balance of these pathways changes, what predictive ability do we have? How do sediment reworking activities affect contaminant transformation (i.e., changes in sediment cohesion, increase in oxidized zones and resulting microbial activity from burrowing organisms? How does recolonization affect contaminant transport and fate? This research can be performed using existing methods.

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29-30 November 1990**

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RISK ASSESSMENT WORKSHOP SYNOPSIS
Glenn Suter II, David Orvos, and Michael Slimak (Co-chairs)
May, 1991

Introduction

Risk assessment originated in the late 19th century insurance industry as a way to estimate risks to human health. At the time, it was defined as "the objectified uncertainty of the occurrence of an undesired event." A century later, risk assessment is applied to many fields yet the definition remains valid. In recent years, the United States Environmental Protection Agency (EPA) has used risk assessment to evaluate hazards and prioritize risks caused by chemicals to human beings. This process remains a critical step in the registration of any new chemical about to enter the commercial market.

In 1983, the National Academy of Sciences (NAS) published a report entitled "Risk Assessment in the Federal Government: Managing the Process." (National Research Council, 1983). This document provided guidelines for assessing risk and quickly became the accepted paradigm, or methodology for assessing risks to human health. Although there can be variations in the sequence of steps, a risk assessment involves several basic analyses, including: a definition of the hazard; an assessment of the degree of exposure to the hazard; an evaluation of the effects resulting from exposure; and a characterization and quantification of the risks associated with the hazard. In each step, uncertainties are associated with various assumptions and the final risk characterization includes a numerical estimate with an associated level of uncertainty.

Based on risk assessments to human health, the EPA and other risk assessors have been working to apply risk assessment to environmental problems. In 1987, the Society of Environmental Toxicology and Chemistry (SETAC, 1987) defined ecological risk assessment as "...the process of assigning magnitudes and probabilities to an adverse effect resulting from human activities or natural catastrophes." The importance of establishing a widely accepted methodology for performing ecological risk assessment stems from our need to evaluate potential ecological problems resulting from hazards such as pesticides, toxic chemicals, contaminated industrial sites, and phenomena such as acid rain, coastal degradation, and climate change.

In February 1991, the National Academy of Sciences held a workshop in Airlie, Virginia to consider the applicability of risk assessment to non-human systems. The NAS workshop participants concluded that the human risk assessment paradigm outlined in 1983 also was appropriate for assessing ecological risk. (NAS, in press).

The relevance and feasibility of ecological risk assessment in the Chesapeake Bay watershed was the subject of the May 6 - 7, 1991 workshop in Solomons, Md. The Chesapeake Bay Ecological Risk Assessment Workshop was the fourth and final event in a series of workshops devoted to the toxicology problems associated with the Bay and was sponsored by the Chesapeake Research Consortium (CRC) and the Scientific and Technical Advisory Committee (STAC) of the Chesapeake Bay Program. Focusing on the ecological risks posed by toxic chemicals, the workshop set out to address the existing risk assessment paradigm and to evaluate its relevance to the Chesapeake. In addition, workshop participants were asked to define research that could contribute information needed to effectively assess ecological risks in the Bay region.

Background

Ecological risk assessment involves a series of considerations meant to evaluate potential harm to specific resources. The process involves several steps, but essentially it identifies resources at risk, specifies the causes of harm to those resources, and evaluates the likelihood of an adverse effect. In ecological risk assessment, the resources at risk can include environmental assets such as fishery stocks, submerged aquatic vegetation, or water quality. The health of these resources can be defined by measurements called endpoints. Risk assessment requires endpoints to be clearly identified and the risks to those endpoints quantified within an acceptable range of uncertainty.

A major difficulty of ecological risk assessment stems from the problem of defining proper indicators of environmental health. Risk assessment implies that the assessor clearly identifies what is at risk. This process is referred to as endpoint selection. The *assessment endpoint* is usually a resource of concern to the public such as a commercially important fish stock, or a population of endangered species. Since it can be difficult to accurately measure the actual commodity, such as the status of naturally fluctuating stocks, often risk assessors must select endpoints that can be measured more easily, and then used as indicators to infer the status of the assessment endpoints. For example, the reproductive health of a small sample of fish can be measured and then used to indicate a deficiency in the population. Such measurable entities are referred to as *measurement endpoints*. The workshop participants outlined important steps to take in assessing risk based on the paradigm put forth by the N.A.S.

1. **Define the Hazard** - Define the hazard to be assessed by choosing endpoints, identifying the sources, and characterizing the reference environment.
2. **Assess Exposure** - Assess the exposure of the organisms of interest to the contaminant of interest. This will include an assessment of the transport and transformation dynamics of the contaminant.
3. **Assess Effects** - Assess the induction of effects as a function of exposure (e.g., dose-response relationships).
4. **Characterize Risk** - Characterize the risks resulting from the estimated exposure and exposure-response relationship and quantify the uncertainties in the data and models.

Risk assessment includes both a numerical estimate of risk and an analysis of associated uncertainty. Uncertainties occur at all stages of the assessment and their characterization should accompany the assessment results. Once each of these steps has been addressed, the results of the risk characterization are communicated to risk managers who incorporate financial, sociological, and political considerations to make decisions regarding curtailment of the risk.

Research Strategy

Participants in this workshop agreed with those in the earlier toxicological workshops on the importance of coordinated multi-disciplinary research. This approach is designed to provide multiple lines of evidence from laboratory and field data, and should integrate biological, chemical, physical, and toxicological information from a series of organizational levels. By integrating this information into an overall ecological framework, the cause and effect relationship, and the severity of the effect can be characterized most accurately. Projects should be coordinated both in subject matter and timing. For example, water quality measurements and contaminant analyses should accompany toxicity testing, biochemical studies, or biological surveys. Such an approach is necessary so that risk assessors can be certain of the associations among measurements.

Modeling may provide an effective tool for determining risk. A generic Chesapeake Bay food chain model may be useful to estimate food chain bioaccumulation and the propagation of effects through the food web. Similarly, a causal-tree model may be used to work back from an effect through intermediate causes to ultimate causes such as releases of various toxicants. It is important to evaluate all plausible causes and all potentially important effects. Key species and contaminants to be included in such models might be taken from the recently published Toxics of Concern List and Habitat Requirements for Chesapeake Bay Living Resources.

In addition to food chain models, population models also are needed for important resource species and sensitive indicator species (e.g., a representative piscivorous bird). Alternate demographic and individual-based approaches should be developed. Individual-based population models capture seasonality of life history processes, and integrate effects of combined toxic materials, harvesting, etc., and other possible compensatory and depensatory mechanisms.

Each risk assessment requires that appropriate spatial and temporal scales be defined. While large scale ecosystem models can offer insight into regional problems, smaller watershed models can provide more realistic risk assessments of chemical hazards. For these reasons we need to develop regional databases for important ecological subregions of the Bay.

Research Needs

The risk assessment workshop panel has identified the following research needs.

1. Define Endpoints

We need to define pertinent endpoints beyond those based on production of commercially important resource species which are often difficult to measure. Endpoints should be measurable or estimable, and related to social, economic, or political interests such as fish stocks and water quality. Examples of potential endpoints include species survival and fecundity and the diversity of species in a given community.

2. Document Sources

We need to document sources of contaminants. The types and amounts of different chemicals entering the Bay from various sources should be quantified and organized into a data base. In this way, we will improve and expand contaminant loading inventories. In order to do this, we need improved analytical methods to assess contaminant concentration in various matrices. To minimize costs, we should focus our efforts on contaminants listed in the "Toxics of Concern" put forth by the Chesapeake Bay Program (EPA, 1991).

3. Investigate Exposure

We need to clarify the routes of exposure of organisms to toxic chemicals. This clarification is clearly a multi-disciplinary problem.

- ***Determine Bioavailable Fractions***

We need to develop methods for defining concentrations of bioavailable forms of important contaminants in different media. Because contaminants that are not available to organisms do not pose a direct risk, concentration measurements need to better approximate bioavailable concentrations. Models should be developed to estimate the bioavailable fractions from measures of total concentration under various physical/chemical conditions.

- ***Develop Models of Transport and Fate***

Better transport and fate models also should be developed. We need more research on cross-media transfers of classes of toxic chemicals across air-water, air-soil, and soil-water boundaries.

4. Assess Effects

To pose a risk, a chemical must be capable of eliciting some detectable effect on an organism. Much progress has been made in developing methods to detect effects of contaminants on organisms, yet there is still much work to be done before we understand the complex yet crucial interactions between chemicals and organisms. We need to develop the tools that enable us to link cause and effect when assessing risk retrospectively, that is, when we need to determine the cause of adverse biological events that have already occurred.

- ***Assess Combined or Synergistic Effects***

Though the effects of many individual toxicants are well understood, contaminants are likely to occur together, and in different environmental conditions. We need to understand the toxic effects of combinations of chemicals that are likely to co-occur at significant concentrations. To do this, we need to assess toxic effects, using both singular and multivariate experimental designs, and incorporate various environmental conditions (e.g., various temperatures, salinities, and life stages).

- ***Estimate Effects of Variable Exposures***

In many environments, exposure to toxic chemicals may not be continuous, and may vary due to episodic processes such as heavy rainfall or industrial timetables. Under these conditions, exposure may occur as a series of intermittent pulses followed by periods of relatively uncontaminated conditions. We need to develop methods to estimate the effects of pulse and variable exposures on living organisms.

- ***Develop Extrapolation Methods***

Often, the endpoints of interest (assessment endpoints) cannot be measured directly and must be determined by extrapolating from other endpoints that can be measured (measurement endpoints). We need to refine methods for extrapolating from standard measurement endpoints to assessment endpoints in order to assess risk effectively.

- ***Conduct Multi-Species Toxicity Tests***

Toxic effects on a given species of organism may be either compensated for or magnified by the presence of other species. We need to investigate the effects of contaminants on multi-species systems to understand the processes involved in the natural environment. Effects should be assessed experimentally using a hierarchy of exposure systems such as those employed in the SAV research effort (Kemp et al. 1980). Also, microcosms or mesocosms that can be moved to various locations for in situ or ambient studies should be utilized.

- ***Designate Standard Indicator Organisms***

A suite of standard organisms should be used as indicators of stress conditions in Chesapeake Bay habitats. To develop a representative hierarchy, we should identify those "indicator" species that characterize different habitats and their respective trophic levels. Standard tests should be developed for each of these indicator species. Moreover, to better understand the life history dynamics of these organisms, we should expand our knowledge of basic Chesapeake Bay ecology.

5. Data Management

- ***Develop a Data Organization System***

We need to develop a system for compiling and organizing physical, chemical, and biological data from the Bay in a way that makes it easy to explore, develop, and display spatial/temporal patterns and associations. It is crucial that the data be organized in a regional framework so that information on selected subregions of the Bay can be acquired easily. This organization system may also include setting up a clearinghouse of information on ongoing research.

- ***Refine Statistical Procedures***

New procedures for statistical analysis should be investigated and developed including methods for distinguishing effects in the field where replication and randomization are not possible. These also should include methods for distinguishing events such as spills, or interventions such as waste treatment, on ecosystem characteristics.

- ***Expand Monitoring Program***

To verify and calibrate models and to quantify and characterize uncertainty we need more toxics data. Therefore, we should refine the monitoring program to collect the appropriate toxics loading data.

6. Modeling

There are many applications for modeling exercises in the risk assessment process. Since we cannot measure the exact concentrations of the thousands of potential contaminants at every point within the Chesapeake Bay, we need to utilize models of contaminant fate and transport to estimate the distribution of contaminants. Similarly, ecological effects models should address ecological processes which can not be addressed directly, and they should be capable of making predictions for the future. Finally, we need risk models to integrate exposure and effects information in order to assess the risk to the resource of interest.

- ***Expand Fate and Transport Models***

Exposure models are needed to convert the spatial-temporal dynamics of contaminant concentrations into exposure of spatially and temporally dynamic endpoint populations. To best assess exposure, we need to integrate chemical fate and transport models with the seasonal and geographic distribution patterns of organisms.

- ***Improve Population Models***

We need to understand how effects of contaminants on individual organisms are propagated through populations and communities. This need should be addressed through population and ecosystem modeling. Models help us estimate the effects of toxicity on higher ecological levels, but they will also clarify the information gaps and drive future research and monitoring programs.

- ***Develop Risk Models***

To estimate uncertainty and to characterize the spatial and temporal distributions of risk, we need to integrate models and data for both exposure and effects. New models need to be calibrated and validated and will require new toxics data. For the future, robust risk models should drive data collection in the monitoring program.

- ***Develop Regional Models***

Regional models simulate the linkages among media, processes, and human activities on large scales. Simple mechanistic models will help us link cause and effect. Even if we do not have enough data to fully calibrate and validate models, conceptual models will help us organize information, structure relationships, articulate key assumptions, and identify data gaps. Needs for more data subsequently will help refine future monitoring programs.

7. Risk Management

- ***Improve Communication***

We need to develop methods to better characterize and communicate risks to managers

- ***Develop Ecosystem Valuation Methods***

We need to develop better and more complete estimates of ecosystem values for cost-benefit analyses.

8. Perform an Ecological Risk Assessment

To get a better idea of what is needed for ecological risk assessment and to better understand the theory behind it, the Chesapeake Bay community should attempt to carry out an assessment. Even before we have collected all the data, finalized all our models, and established clear cause and effect relationships between toxic chemicals and living resources, we should move forward with a risk assessment on an important Chesapeake Bay species or an identified hazard. This will help to identify data gaps, quantify uncertainties, refine procedures, and shape our assumptions; all of which is required before we routinely apply risk assessment to a toxic contamination problem.

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Appendix A

Ambient Toxicity Workshop, July, 1989

Suborganismal Workgroup Session Report

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. John Pritchard, Dr. Wolfgang Vogelbein, and Dr. David Wright; submitted by Dr. Jay W. Gooch, suborganismal plenary session speaker and convener.

This document was prepared based on comments from investigators with research experience in suborganismal responses. These researchers gathered at the Ambient Toxicity Assessment Workshop and their comments and suggestions relate to the use of biochemical effects measurements for the assessment of ambient toxicity in Chesapeake Bay waters.

The general consensus 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 distribution 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 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 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 in 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 contaminants.

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