Proceedings and Summary of the Workshop on Finfish as Indicators of Toxic Contamination

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CONTENTS

1. OVERVIEW OF THE NATIONAL ESTUARY PROGRAM AND OBJECTIVES OF THE WORKSHOP ON 'FINFISH AS INDICATORS OF TOXIC CONTAMINATION

In 1985, the U.S. Environmental Protection Agency (EPA) initiated the National Estuary Program. The program was designed to protect and restore water quality and living resources in the nation's estuaries. Under the authority of the Clean Water Act, the program establishes working partnerships with other Federal agencies, state and local governments, academic and scientific communities, industries and businesses, public organizations, and private citizens. The goal of these working partnerships is to address collectively the environmental and/or management problems of estuaries. The national program, which is administered within EPA by the Office of Marine and Estuarine Protection (OMEP), seeks to

- o increase public understanding of the nature of estuaries and their environmental and management problems;
- o provide state and local managers with the best scientific and technical information available;
- o transfer technical and management expertise and practical experience to state and local governments;
- o increase understanding of both the need for area-wide or basin-wide planning and its benefits;
- o develop plans to control pollution sources and restore living resources; and
- o gain acceptance of the public and private costs of increased pollution controls and estuarine restoration.

Current estuary programs include the Buzzards Bay (MA), Narragansett Bay (RI), Long Island Sound (NY, CT), Puget Sound (WA), Albemarle/Pamlico Sounds (NC), and San Francisco Bay (CA) programs.

In each estuary program, the EPA regional office(s) works with program participants to define the problems of the estuary and to reach agreements to reduce the causes of point source pollution and the polluting effects of other human activities contributing to these problems. Estuary programs may establish goals to maintain currently existing conditions, to restore a selected level of water quality or living resources, or to maintain pristine conditions within an estuary. Population growth and its associated increasing and conflicting demands for water uses can cause participants of estuary programs to reexamine and refocus their existing programs and to develop new initiatives that adequately protect the estuary.

The principal goal of each estuary program is to produce a comprehensive Master Environmental Plan that describes actions to control point and non-point sources of pollution; to manage and protect living resources; to implement sound land use practices; to control freshwater input and removal; and/or to establish anti-degradation policies for pristine areas. To be effective, this plan must identify the parties responsible for these actions, and the revenue sources necessary to do the job. The plan also must provide for monitoring of environmental quality in the estuary, periodic program review, program redirection in response to new problems or information, and a mechanism to resolve conflicts among participants.

In regional estuary programs, ambient environmental quality should be monitored primarily to set priorities for pollution control and to verify the success of management strategies implemented under the Master Environmental Plan. In addition, the National Estuary Program needs to determine whether indicators of finfish health can serve as warnings of toxic contamination, and can thus assist in setting priorities among estuaries for inclusion in the program. To

successfully conduct such a trend-monitoring program, indicators of environmental quality that are both scientifically appropriate and cost-effective must be identified and/or developed. The Workshop on Finfish as Indicators of Toxic Contamination will partially fulfill this need by identifying a set of appropriate indicators of toxic contamination for assessing potential human health and ecological concerns.

OMEP sought scientific input to assist in an evaluation of the many possible finfish indicators of toxic contamination to determine which indicators may be appropriate, at present, as estuarine monitoring tools. Therefore, the purpose of the workshop was to systematically organize and set a priority ranking of a list of methods, for use of fish as indicators of the health of estuaries both emphasizing those most useful to immediate management needs and identifing those that show the most promise for future development.

2. SUMMARY OF THE OPENING PLENARY SESSION

Dr. Tudor Davies, Director of EPA's OMEP opened the workshop. He reviewed the current structure of most EPA regulatory programs, many of which are media-based (i.e., programs intended to address only specific environmental media, such as air, land, or water). Permit limitations for toxic pollutants typically are technology-based, with limited testing of effluent toxicity and modeling of wasteload allocation. Monitoring, therefore, is typically oriented towards assessing either effluent pollutant loads or ambient concentrations of pollutants for which wasteload modeling can be conducted, only recently has the need for ambient biological monitoring received increased attention in EPA's surface water programs. The National Estuary Program, in particular, is seeking to develop and apply a set of scientifically appropriate and cost-effective indicators of estuarine environmental quality as ambient monitoring tools. This workshop's goal is to assess the extent to which finfish indicators can serve this purpose.

Ms. Michelle Hiller, Chief of OMEP's Technical Guidance Branch, then summarized the goals and objectives of EPA's National Estuary Program and described how finfish indicators of toxic contamination may be used within the program. As one potential use, EPA wants to determine whether such indicators can assist in selecting estuaries to be included in the national program by serving as warnings of serious toxic contamination that either threatens or occurs in an estuary. In addition, indicators are needed within estuaries once the estuaries have been included in the National Program. They are needed to help determine where the most severe biological effects are occurring; the spatial extent of critical impacts; where possible, the specific pollutant or pollutants most responsible for critical biological impacts; and the success of toxic pollutant abatement strategies implemented for an estuary.

The workshop moderator, Dr. Gary Petrazzuolo, Technical Resources, Inc., then described the subjects of each working group for the initial discussion and ranking of indicators. These subgroups, based on the participants' areas of expertise and interest, were

- o Anatomic Pathology
- o Immunology
- o Bioaccumulation and Enzymes
- o Reproduction and Development,
- o Physiology, Behavior, and Population.

Each of the subgroups was to develop a list of indicators, to consolidate and organize the list by merging closely related methods, and to characterize each of the indicators. Indicators were characterized by considering their usefulness for identifying effects of toxic contamination to fish, the ecosystem, and/or human health, along with the following set of characteristics:

- o biological significance (i.e., there is a widely accepted cause-and-effect relationship between toxic pollution and the indicator, or there are either few or no conflicting plausible explanations for the observed effect other than toxic pollution);
- o cost-effectiveness;
- o availability for widespread use; and
- o applicability, including their use as
  - early or late indicators (i.e., sensitivity -- the effect will appear following acute or chronic exposures);

- pollutant-specific indicators (i.e., those that can indicate exposure to a specific pollutant or class of pollutants);
- species-specific (i.e., those that can be applied only to a few or one species of fish); and
- spatially-restricted indicators (i.e., those that are only useful on small spatial scales).

The final objective of each subgroup was to rank the identified indicators. The ranking was to be based only on the first three criteria (biological significance, cost-effectiveness, and availability). Information on the various aspects of applicability was reported, but did not necessarily indicate greater or lesser usefulness of a given indicator. Ranking was to be accomplished by asking each subgroup to provide lists based on the four following ranking methods:

(1) identify the best indicator on the list;

(2) identify the best one-third of the indicators from the list;

(3) rank each of the indicators in order of its importance,and

(4) rank each indicator as either "good" or "bad."

Dr. Petrazzuolo noted that the technical background paper, prepared for the workshop by Dr. Margaret McFaden-Carter of the University of Delaware, provided a starting point for each subgroup's discussion. This paper (see Appendix E) surveys the available indicator methods. Dr. Petrazzuolo then opened the plenary session to questions and asked whether the participants felt any major categories of indicators had been omitted from the background paper. No such categories were identified in the opening session. (However, additional indicators were later identified and evaluated by the four subgroups).

3. SUMMARY OF THE PLENARY SESSION ON SUBGROUP REPORTS

Following the subgroup sessions, all workshop participants again met in a plenary session to summarize and discuss each subgroup's findings. (See the individual subgroup summaries for a more detailed description of these discussions.) The most highly ranked indicators from each subgroup are presented in Table 1. A glossary of the indicator methods follows in Table 2.

Workshop participants were asked to choose the best indicators from those by the subgroups considered. Several participants questioned whether the group, as a whole, was capable of making such an evaluation. A lengthy discussion followed in which participants presented possible scenarios for using various sets or groupings of toxic pollution indicators. To clarify the technical questions being asked, an EPA representative described the major stages of each estuary program's data analysis in detail. The stages are

- problem definition,
- characterization, and
- design and implementation of a monitoring program.

To screen estuaries for incorporation into the National Estuary Program, a process analogous to the first phase of an individual program (problem definition) is used. Then individual, regional programs carry out all three steps to define problems, to develop management and abatement strategies, and to monitor recovery. Different indicators or sets of indicators could be used during each of these phases. Workshop participants agreed to evaluate the indicators according to their usefulness in each of these phases of an estuary program.

TABLE 1. SUMMARY OF SUBGROUP RANKINGS OF FINFISH INDICATORS

SUBGROUP	RANK OF METHODS
Anatomic Pathology	1. Gross changes 2. Ordinary histological methods 3. Ultrastructural histology
Bioaccumulation/Enzymes	
a. Bioaccumulation	1. Residue levels 2. Models 3. Metabolite profiles
b. Enzymes	1. Induction (e.g., mixed function oxygenases and metallothionein) 2. Inhibition (Acetylcholinesterase and gill ATPase) 3. Blood chemistry (clinical)
Reproduction and Development, Physiology, Behavior, and Population	
a. Reproduction/ Development	1. Cytogenetics 2. Larval development and viability 3. Embryo viability
b. Physiology	1. Hematology (blood chemistry) 2. Swimming stamina
c. Behavior	1. Avoidance/attraction 2. Abnormal behavior
d. Population	1. Spatial distribution profile 2. Abundance 3. Age structure profile
Immunology	1. A triad of macrophage indicators (phagocytosis, chemiluminescence, and killing ability) 2. Experimental measures of disease resistance 3. Jerne plaque assay

TABLE 2. GLOSSARY OF FINFISH INDICATOR METHODS

Anatomic Pathology

1. Gross changes--this category includes obvious abnormalities such as fin erosion, skeletal deformities, tumors, etc.

2. Ordinary histological methods--observations that can be made using standard light microscopy techniques are included in this category.

3. Ultrastructural histology--these techniques generally require the use of scanning or transmission electron microscopy.

Bioaccumulation

1. Residue levels--this term refers to the analysis of tissues for contaminant levels of some or all of the 129 priority pollutants, or other contaminants of local concern.

2. Models--mathematical models can be used to predict the bioaccumulation potential of a particular compound or group of substances based on physical, chemical, and structural information.

3. Metabolite profiles--many organic compounds are metabolized by finfish. These metabolites are not included in standard residue analyses. Because some metabolites bind tightly to tissue macromalecules, information on contaminant metabolites may be a better indication of past exposure history than information on residue levels of the parent compounds.

Enzymes

1. Induction--certain enzymes or proteins may be produced by an animal as a result of exposure to a xenobiotic. Mixed function oxygenases (MFOs) are enzymes that oxidize some non-polar organic compounds to more hydrophilic forms. Metallothioneins are proteins that bind to certain metals. Specific MFOs or metal binding proteins may be produced as a result of exposure to a particular contaminant.

2. Inhibition--particular enzymes or physiological processes may be inhibited by exposure to contaminants and, therefore, such a response may indicate a deleterious effect.

3. Blood chemistry--a number of measurements of blood chemistry are performed routinely in clinical diagnosis, and may be adapted to diagnosing pollutant stress in fish, They include both the levels of certain substances (e.g., glucose, cholesterol, triglycerides, albumin) and enzyme activities (e.g., alkaline phosphatase, lactate dehydrogenase).

TABLE 2. GLOSSARY OF FINFISH INDICATOR METHODS (Continued)

Reproduction/Development

1. Cytogenetics--the study of the relationship between chromosomal aberrations and pathological conditions.

2. Larval development and viability--includes such endpoints as growth rates, percent abnormalities and mortality.

3. Embryo viability--for many fish species this is the most sensitive stage in their life history. The endpoints that are commonly employed include development rate, type and extent of developmental abnormalities, hatching success, and mortality.

Physiology

1. Hematology--the study of the blood. This includes the same types of clinical blood measurements described above.

2. Swimming stamina--this is a measure of the general fitness of a fish as determined by its ability to swim against a current.

Behavior

1. Avoidance/attraction--changes in these behavioral attributes are measured by comparing the reactions of fish before and after exposure to the test material.

2. Abnormal behavior--in addition to avoidance/attraction behavior, other behavioral changes (e.g., erratic swimming, lethargy) indicate specific modes of toxicity (e.g., neurological or metabolic dysfunction).

Population

1. Spatial distribution profile--analysis of the spatial distribution of a species of fish within the estuary. Distribution may be directly related to avoidance/attraction behavioral changes.

2. Abundance--a relative measure of population demographics.

3. Age structure profile--a skewed age structure profile may be indicate an unstable population.

TABLE 2. GLOSSARY OF FINFISH INDICATOR METHODS (Continued)

Immunology

1. A triad of macrophage indicators (phagocytosis, chemiluminescence, and killing ability).
    a. phagocytosis--a measure of the ability of macrophage cells to engulf microorganisms, other cells, or foreign particles.

    b. chemiluminescence--the relative chemiluminescence of macrophages may correlate with the degree of exposure to xenobiotics.

    c. killing ability--a measure of the ability of macrophage cells to kill microbes or tumor cells.

2. Experimental measures of disease resistance--by injecting disease microorganisms into feral fish, the relative disease resistance of individuals or populations can be determined.

3. Jerne plaque assay--B-lymphocyte measurement of antibody production.

The problem definition phase was divided into an initial qualitative step (called "problem identification") and a later, more quantitative process (called "screening"). Problem identification determines initially whether an estuary should be considered for toxic contamination screening. Problem identification may be systematic (i.e., part of an on-going data collection) or anecdotal. This initial step involves minimal new expenditures. The workshop participants agreed that the following finfish indicators would be useful during this phase:

- o gross behavioral changes,
- o anatomical changes,
- o population changes, and
- o tainting of commercial fish.

During the cross-estuary screening phase, scientists will determine whether fish are significantly stressed and whether toxics are a likely cause. The workshop generally agreed that, during this phase, the following indicators of toxic stress would be most appropriate, on the basis of their relatively low cost and general applicability:

- o nonpollutant-specific indicators (immunological, i.e., blood samples for hematocrit determinations and kidneys for observation of macrophage phagocytes, chemotoxins, and assays of estuarine waters with mortality as the indicator);
- o indicators of synthetic organic effects (e.g., cytochrome P-450 and assays of liver for metabolites);

- o indicators of toxic metal effects (e.g., metallothionein assays and/or metal residue measurements in liver tissues); and

- o pesticide analyses (where there is reason to believe agricultural activities are a source of pollution).

However, a few participants thought that the indicators used in the screening phase should not be restricted to biochemical types, but should also include indicators at the organismal and population levels (e.g., gross pathology, abundance, and distribution). They also agreed that it would be important during this phase of the program to archive samples (i.e., brain, gill, liver, kidney, flesh, and spleen) for subsequent tests or analyses. If it was determined that there may be toxic contamination, then further histological or residue analyses would be warranted.

As part of the characterization process for an individual estuary, a synthesis of historical data should be performed to further define problems and to identify data gaps. This synthesis would include data on pollutant loads, ambient water and sediment conditions, and any data on biological indicators that may already exist. The indicators selected for the characterization phase also should be useful for distinguishing possible causes of the toxic stresses observed in the estuary whenever possible. It was hoped that this information would both assist in the process and perhaps help to defend the specific pollutant abatement actions for an estuary.

After discussing potential characterization indicators, the participants decided to divide characterization into two determinations: (1) a determination of the nature, severity, and extent of impacts on fish populations; and (2) a determination of cause-and-effect relationships between specific pollutants or types of pollutants and major observed impacts. To determine the nature, severity, and extent of population impacts, the following indicators were identified:

- o cytogenetics,
- o egg and larval development and viability,
- o histopathology (within tissue, sublethal effects),
- o immunological (triad of macrophage tests), and
- o enzymes (e.g., mixed function oxygenase (MFO) system;(metallothionein).

To show cause-and-effect relationships, the workshop agreed it would be necessary to conduct these same tests under controlled laboratory conditions. The techniques used during the first phase of characterization could be modified for use in a laboratory to include ambient water or sediment test phases and pure compounds. However, workshop participants noted that several highly pollutant-specific indicators have been developed under laboratory conditions and now are ready for field testing. Other such indicators will be ready in the next 1 to 2 years. Using such methods, it might soon be possible to more effectively trace the causes of some impacts observed in the field. Participants also suggested that certain ultrastructural analyses, while expensive, could be used for evaluating pollutant-specific causes of stress.

Monitoring ecosystem recovery, and the question of suitable indicators for this purpose were then discussed. Participants agreed that the indicators previously listed should be used again to compare recovery on a site-specific basis. For a system-wide, long-term monitoring program (i.e., to be conducted over a period of 5-20 years), the workshop was then asked if finfish indicators should have a role and, if so, what should it be? Participants agreed that the following indicators would be beneficial for such a monitoring program:

- o population studies,
- o residues,
- o reproductive success (including embryo viability), and
- o histological markers (sublethal effects).

The workshop generally agreed that finfish should be part of a long-term monitoring program and that finfish indicators would be useful for monitoring recovery.

Finally, at the plenary session, the participants were asked to identify finfish indicators relevant to human health. They identified tissue residues as the only unequivocal finfish indicator of human health impacts (toxic, mutagenic, and carcinogenic). Metabolites were considered a subset of residues.

Each subgroup was asked to identify the most promising methods, of those they considered, that are presently available and those that need further research and development. Availability was considered as (1) immediate with widespread usage, (2) immediate with only limited current usage (0-1 year), (3) short-term (1-2 years), and (4) long-term (3-5 years). A summary of the subgroup conclusions follows:

- o Immediately available and in widespread use
  - gross pathology
  - some histopathological indicators
  - tissue residues
  - egg and larval viability and development
  - population techniques

- o Immediate availability but not in widespread use (0-1 year)

  - metallothionein
  - cytochrome P-450 (and other mixed function oxygenase system enzymes)
  - individual macrophage triad tests

- o Short-term availability, with some development and/or field baseline data needed (1-2 years)

  - macrophage triad (phagocytosis, chemiluminescence, and killing ability)
  - adducts (DNA and synthetic organics)
  - bioaccumulation models
  - cytogenetics
  - hematology
  - ultrastructural pathology
  - combination of macrophage, T-cell, B-cell, and disease resistance methods

- o Long-term availability, with methods needing further research and development (3-5 years)

  - blood chemistry (enzymes)
  - histochemistry
  - development of inbred fish lines to support development of immunological assays
  - in vitro tests of several immunological indicators

PLENARY SESSION CONCLUSIONS

While trying to agree on a set of finfish indicators, workshop participants made several recommendations and comments concerning the

use of finfish as indicators of toxic contamination. The workshop participants agreed that no single test was adequate. Instead, they considered a suite of tests necessary to characterize toxic effects. In addition, the workshop maintained that the suite of indicators should be field tested simultaneously in fish collected from several study areas to compare the sensitivity of indicators across major estuarine categories. Participants also stressed the need to study a "pristine" reference estuary or set of estuarine areas. The purpose of these studies would be both to provide baseline data for comparison to stressed systems and to determine the normal ranges of the recommended finfish indicators under field conditions.

Workshop participants also stated that finfish indicators may not be good screening tools for cross-estuary evaluations. Participants indicated that more easily interpreted endpoints can be evaluated to assess whether or not a system is stressed. Also, the use of finfish as indicators in trend monitoring programs within estuaries may be limited because some key methods have not yet been field verified and because other, less mobile organisms (e.g., certain benthic species) may be better suited as indicators. However, studies using less mobile species also may be limited inasmuch as sampling heterogeneity can be more pronounced on a local scale if pollutant distribution is patchy. Mobile organisms, therefore, may actually be better suited at integrating toxic contamination burdens for meso- or regional-scale assessments. Furthermore, EPA representatives noted that there are regulatory and programmatic needs for resource management agencies to evaluate indicators of impacts on fish.

The workshop generally agreed that behavioral, enzymatic, and immunological effects would appear earlier (i.e., following shorter toxicant exposure) than histological or gross anatomical changes. Therefore, these effects would be better suited as early indicators of toxic contamination.

The workshop also pointed out that it is currently difficult to make cross-technique assessments for those methods now used routinely

and that it would be valuable to provide longer-term research support to develop methods for use in the field. In addition, more detailed information should be obtained on both what techniques are currently available and which institutions have the capabilities to perform them.

4. SUMMARY REPORT FROM THE SUBGROUP ON ANATOMIC PATHOLOGY

Although death of fish has historically been used, and currently is used often as the primary indicator of problems related to toxic contamination, this subgroup suggested using a series of sublethal effects occurring in a progression that may end in death to describe and evaluate indicators of toxics in finfish (Figure 1). This progression includes early changes that are usually reversible, late changes that are usually irreversible, and intermediate changes that may or may not be reversible. It was suggested that the discussion of indicator methods identify the stage(s) in this progression where the methods would be applicable. Approaches to the use of pathological indicators may be organ-specific, manifestation-specific, or systemic (or holistic).

For the organ-specific approach, with organ defined as a morphologically discrete and functionally organized anatomical component, examples of changes include the following:

- o Gross

  External: discoloration, skeletal deformities, fin rot, skin ulcerations, hyperemia, eye lesions (opacities), neoplasia, parasites, edema

  Internal: edema, (including ascites), organ displacement, neoplasia, parasite

- o Histological (tissue)

  Classes of changes: inflammatory, cellular alteration, hyperplastic/neoplastic, melanomacrophage aggregation increase, parasitic

- o Ultrastructural (subcellular)

  Classes of changes: Inclusion bodies, lysosomal, parasitic, smooth endoplastic reticulum changes

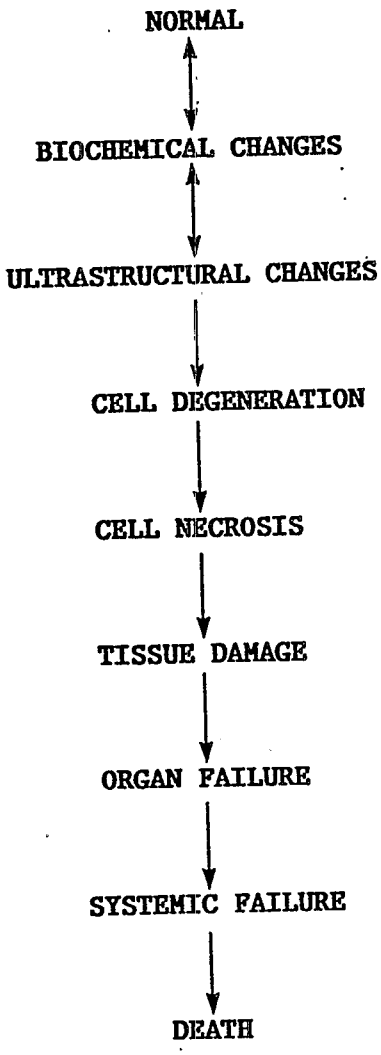

Figure 1. Progression of Indicators of Toxic Stress

- Histochemical

  Enzymatic: technologies to be explored include differential staining for glutamyl transferase, other enzymes, ion-specific assays, and microprobes.

Using the manifestation-specific approach, the following manifestations have been associated with the following kinds of toxic contamination and other stresses. This listing is not all-inclusive. Additionally, in every case the effects of chemical mixtures in synergy and opposition must be considered and may be indicated by

- Fin erosion--PAHs, PCBs, ammonia, nitrites, water-soluble hydrocarbons, trauma

- Skin ulceration--PAHs, PCBs, parasites, trauma/injury, sunlight (UV radiation)

- Eye disease--PAHs, phthalate esters, nutrition, parasites

- Gill disease--ammonia, nitrites, metalo-organics (tin), organics, PAHs, metals (Cd), parasites

- Liver neoplasms--PAHs, PCBs, chloramines, nitrosamines, aflatoxins, metals (Cr, Cd), parasites

- Skeletal deformities--organochlorines, herbicides (trifluralin), insecticides (organophosphates), metals, nutrition

- Papillomas--viruses (from contamination?)

- Pancreatic diseases--PAHs, nitrosamines, viruses

- Kidney diseases--heavy metals (Hg, Pb, Cd), nutrition, nitrosamines, parasites

- Gastrointestinal tract diseases--PCBs, petroleum hydrocarbons, nitrosamines, viruses, bacteria, parasites.

The multiple system or holistic approach includes all parts of the organism and all manifestations. For example, ammonia has been associated with effects on gills, skin, gastrointestinal tract, and aggregate macrophage changes. Industrial organics have been associated with effects on brain and liver.

The indicators of pathology discussed by this subgroup were gross changes, histological, ultrastructural, and histochemical effects. Although all the indicators were considered useful for identifying toxic contamination in finfish, only gross changes were useful indicators of toxic contamination at the ecosystem level. The value of the other indicators at the ecosystem level was uncertain or unknown. Cause-effect relationships may be implied by associating a syndrome of effects with likely causative agents. None of the indicators was considered valuable in assessing effects on human health. The subgroup discussed and evaluated the methods for assessing these indicators. Table 3 summarizes the results of this process. A brief summary of the subgroup's discussion on indicators at all levels of pathology follows.

GROSS CHANGES

As indicators of toxic contamination, gross changes signal a very disturbed ecosystem. The methods for assessing gross changes are cost-effective, widely available, and easily taught. It also is of low sensitivity. In terms of the progression shown in Figure 1, gross pathology can identify organ failure and system failure, i.e., where the process is usually irreversible. This methods is not pollutant-specific, but is specific for space and time. The methods is ready to be used with many species (i.e., it is not species-specific).

HISTOLOGICAL EFFECTS

Histological effects may be indicators of minimally disturbed environments. Histological methods can detect subanatomic manifestations, at early to intermediate stages in disease progression, i.e., in the transition area between reversible and table 3 here irreversible changes (See Figure 1). These methods can be performed cost-effectively by high-production laboratories, although the availability of such laboratories may be limited. Histological methods also can be used in highly polluted environments, and are specific for time and space. Certain pathological lesions can be used to identify classes of pollutants (heavy metals, aromatic hydrocarbons, polychlorinated compounds, and mixtures of compounds), but these lesions are not specific for individual pollutants. The methods are ready to be used with many species.

ULTRASTRUCTURAL EFFECTS

Ultrastructural effects can be detected by electron microscopy, an expensive method with limited availability. The meaning of ultra-structural changes as an indicator of toxic contamination is not always understood, although they may offer the potential for detecting problems early. For example, proliferation of hepatocytes tends to reflect drug or toxicant exposure within hours of that exposure, and may continue throughout chronic exposure. The detection of ultrastructural changes may also be used as a confirmatory tool: detecting changes in mitochondria, a characteristic response to cyanide, was used to confirm cyanide as the cause of a fish kill in Ohio.

HISTOCHEMICAL EFFECTS

The meaning of histochemical effects as an indicator is unknown, and the methods for their detection are expensive and of limited availability. Histochemical methods may be useful for validating

TABLE 3. ANATOMIC PATHOLOGY: SUMMARY OF EVALUATION OF INDICATORS

CRITERIA	GROSS CHANGES	HISTOLOGICAL EFFECTS	ULTRASTRUCTURAL EFFECTS	HISTOCHEMICAL EFFECTS
Unequivocal meaning	Disturbed ecosystem (highly impacted)	Identify classes of compounds (industrial, municipal, agricultural)	Unknown	Unknown
Cost-effectiveness	High	Yes, with high production labs; Yes, with minimally disturbed environments	Very expensive	Very expensive
Availability	High	Technology available but geographically limited	Very limited	Very limited
Applicability - early/late sensitivity	Very late	Early to intermediate	Potential for early detection	Unknown but has potential
- pollutant-specific	No	No	Unknown	Unknown but has potential
- species-specific	No	No	Unknown	Unknown but has potential
- scope-specific	Yes	Yes	Unknown	Unknown but has Potential

suspected toxic contamination, but they cannot currently be used for monitoring because not enough is known about the significance of their manifestations. The subgroup suggested that if correct enzymatic tests are used for putative foci (e.g., the liver), alterations in metabolism might be detected that could be associated with the development of neoplasms. These methods may be applied to the multi-stage theory of carcinogenesis.

DISCUSSION

It was emphasized that a set of tests, rather than any single test, should be used to identify toxic contamination. Gross anatomical examinations and histologic tests should be performed in conjunction with chemical analyses of water and sediment and residue analyses to determine body burdens of toxics.

Laboratory studies can establish causal relationships, but their applicability to the field must be established. For example, trifluralin effects (vertebral injury and hyperostosis), induced by low concentrations in the laboratory, have been validated with the same lesions in field-exposed, wild populations of fishes. Similarly, PCB-induced liver damage in laboratory-exposed fish has been found in wild, PCB-exposed fish. However, native populations may develop a tolerance to the contamination, which probably would not occur in laboratory or field testing.

The use of tumors as indicators requires evaluating their significance in fish populations. Neoplasms in finfish, including carcinomas, have been associated with contamination by PAHs, PCBs, and heavy metals. The subgroup suggested that if a fish has a visible tumor, it may contain a level of carcinogens that can pose potential health effects for humans ingesting it. Therefore, fish with severe fin erosion (fin rot), integumental ulcerations, and cataracts should not be eaten. Further, an incidence of tumors that is significantly

above expected levels in wild populations of a particular aquatic system indicates that toxicants or carcinogens may be a major cause of stress in the ecosystem.

RECOMMENDED INDICATORS

Based on the evaluation criteria and ranking by the four suggested methods, the anatomic pathology indicators recommended were

- Gross changes (for highly disturbed ecosystems)
- Histological effects (for minimally disturbed environments).

RESEARCH NEEDS

The following research needs were identified for improving the usefulness of anatomic pathology indicators of toxic contamination in finfish:

- Gross changes
  - Conduct field tests to quantify changes across disturbed environments to help assess significance and commonality;
  - Define impacts of fish sampling techniques on gross lesions;
  - Develop reference works on documented pathology and on gross and microanatomy of fishes [It was noted that development of a textbook on gross and microanatomy of fish (identified as a critical need) is underway].

- Histological effects

  - Improve specificity and interpretation in relation to effects;

  - Train fish histopathologists;

  - Standardize vocabulary and interpretation of lesions;

  - Establish baseline data on selected species.

- Ultrastructural effects

  - Conduct basic laboratory research to demonstrate ultrastructural effects from classes of compounds;

  - Conduct research to relate laboratory studies to field studies.

- Histochemical effects

  - Direct basic research to explore use as a monitoring tool.

5. SUMMARY REPORT FROM THE SUBGROUP ON BIOACCUMULATION AND ENZYMES

The subgroup discussion began by selecting a spokesperson (Dr. Jerry Neff, Battelle) and a recorder (Ms. Patricia Fair, NMFS). The group was then asked to rank the major finfish indicator methods that measure bioaccumulation/metabolism of toxicants and changes in enzyme function due to toxic pollution. The initial indicators for each category are ranked below and are followed by brief summaries of the discussion for each indicator:

- o Bioaccumulation
  - Residue Levels
  - Metabolite Profiles
  - Chemical/Physical Models
  - Kinetic Biology-Based Models
  - Rapid Mutagenicity Tests (Ames/Lambda Prophage) on tissues or tissue extracts.

- o Enzymes
  - Enzyme Induction (Particularly Mixed-Function Oxygenases
  - Enzyme Inhibition
  - Blood Chemistry
  - Adaptive Stress Responses

BIOACCUMULATION

Residue Concentrations/Metabolite Profiles

The subgroup ranked residue concentrations and metabolite profiles as the best indicators, according to workshop criteria. These indicators provide a major link to human health effects. Regarding the use of residue levels, the subgroup noted that demersal species, which form localized populations (i.e., have limited migratory ranges), have historically been most useful for monitoring. The subgroup cautioned,

however, that finfish are not always the best sentinel organisms for many types of pollutants because even demersal species are mobile relative to benthic invertebrates. Also finfish metabolize many classes of compounds rapidly. It was stressed that residue levels in finfish should be used in conjunction with other measures of biological effects to establish causal relationships of particular pollutant sources to observed field effects.

The subgroup further noted that for some types of pollutants, metabolite profiles are a better indicator of past exposure than residues. Developing metabolite profiles involves identifying the concentrations, characteristics, and distribution of pollutant metabolites, conjugates, and adducts in various tissues, fluids, and subcellular sites. However, this subgroup also observed that not enough is known about the pharmacokinetics of pollutants and their metabolites in fish to firmly establish exposure history. Not all methods are currently available; this was cited as a topic for continued research.

Chemical/Physical Models

The subgroup ranked chemical/physical models as the next most promising method for assessing the likelihood of stress due to bioaccumulation. Models discussed were primarily those used to predict distribution, environmental behavior, and fate of pollutants in marine ecosystems. The subgroup cautioned, however, that these models cannot be used alone. They must be used in conjunction with biological models, and must be calibrated and verified under various environmental conditions. These models are useful for providing worst-case estimates of bioaccumulation impacts. However, models must be used carefully because they do not provide the conclusive evidence that residue monitoring can provide.

Kinetic Biology-Based Models

These models were characterized primarily as a means to predict and assess food chain transfer of pollutants. These models have potential uses for both predicting ecosystem-wide effects and linking environmental contamination to analysis of human health risk.

Rapid Mutagenicity Tests (Ames/Lambda Prophage)

Rapid mutagenicity tests were ranked as the next most useful indicators of the effects of bioaccumulation and metabolism. The subgroup stated that it is often difficult to interpret these tests in an environmental context, but they may provide a link to human health considerations. These tests still require substantial development because they must be modified for use with tissue/sediment extracts and because they may yield an unacceptably high frequency of false positives or negatives.

Table 4 summarizes the group's evaluations of these indicators according to EPA's criteria. "Unknown" indicates that the subgroup could not determine or agree whether the indicator met the criterion.

ENZYMES

Enzyme Induction (Particularly Mixed Function Oxygenases)

The subgroup ranked enzyme induction as the best indicator in this category. This indicator is highly pollutant-specific for certain classes of organic and metal pollutants. It is particularly useful for detecting responses that are initially adaptive, but which may become maladaptive with toxification/detoxification. These methods are currently being refined and improved to make them more routine.

Mixed-function oxygenase system induction was singled out as a subcategory of indicators specific to polycyclic aromatic hydrocarbons and PCBs. Measurement of specific forms of cytochrome P450 in

TABLE 4. BIOACCUMULATION/METABOLISM INDICATORS

Criterion	Residue Levels/ Metabolite Profiles	Chemical/Physical Biology-Based Models	Rapid Mutagenicity Tests
Unequivocal meaning	Yes	Moderate	Yes
Cost-Effectiveness	Moderate	Yes	Yes
Availability	Moderate	Moderate	Moderate
Applicability -early/late sensitivity	Early	Early	Unknown
-pollutant-specific	Yes	Yes	Yes
-species-specific	No	Can be	No
-scope-specific (spatial)	No	No	No

particular is a very specific and highly sensitive indicator of exposure to particular pollutants. Measurements of other specific enzyme activities (e.g., aryl hydrocarbon hydroxylase, benzo[a]pyrene hydroxylase, etc.) are subject to substantial variability, but are still valuable indicators.

Enzyme Inhibition

The subgroup ranked this category of indicator next most useful because many methods are readily available. However, some assays may need to be adapted specifically for fish tissues. The subgroup also noted that these indicators have varying degrees of pollutant specificity, depending on the particular enzyme systems involved. Also, there are uncertainties about the effects of natural indigenous and exogenous factors on basal enzyme activity. Thus, the subgroup urged caution in randomly applying enzyme inhibition; instead, it should be used as an indication of exposure to specific classes of chemicals. Also, baseline data are needed to establish normal ranges. The subgroup mentioned the following examples of enzyme inhibition:

- Acetylcholinesterase
- Gill ATPase
- δ-Aminolevulinate dehydratase
- DNA-polymerase
- Glucose-6-phosphate dehydrogenase.

Blood Chemistry

The subgroup ranked blood chemistries as the next most promising set of indicators. They were mentioned as a valuable clinical approach. However, the subgroup noted that more baseline data are needed to further the use of this group of indicators.

Adaptive Stress Responses

Adaptive stress responses were noted as an important set of indicators because their meaning is fairly well established, and they hold promise of being cost-effective. However, the subgroup also noted that these responses are non-specific, i.e., any kind of stress will elicit the response. Three examples of potentially useful adaptive response indicators were specifically mentioned--stress protein responses, adrenocortical responses, and neurochemical responses. The subgroup felt that these methods warrant more development; infact, some methods (particularly metallothionein assays that are specific for metals exposure) are in the field testing stage at present.

The subgroup also evaluated enzyme indicators based on the criteria provided by EPA. Table 5 summarizes its findings. Again, "unknown" indicates that the group could not determine or agree whether the indicator met the criterion.

RESEARCH AND DEVELOPMENT NEEDS

The group established the following priorities for the research and development needs of the discussed indicators.

- Further develop and refine immunologic techniques to quantify specific forms of cytochrome P450 (2-3 years development time) to provide a highly specific, inexpensive and easy-to-apply assay;

- Further evaluate the use of stress proteins, particularly metallothioneins, and very low molecular weight proteins, as general- and pollutant-specific indices of pollutant stress (1-3 years);

- Evaluate pollutant specificity of different serum enzyme assays, and develop databases for species of interest;

TABLE 5. ENZYME INDICATORS

Criterion	Enzyme Induction	Enzyme Inhibition	Blood Chemistry	Adaptive Stress Response
Unequivocal meaning	Yes	Yes	Moderate	No, any stress will elicit response
Cost-Effectiveness	Moderate	Yes	Moderate	Yes
Availability	Moderate	Yes	Variable	Yes
Applicability -early/late sensitivity	Early	Unknown	Early	Unknown
-pollutant-specific	Yes	Yes	Yes	No, except metallothionein
-species-specific	No	No	No	No
-scope-specific (spatial)	No	No	No	No

- Develop immunological or spectrofluorometric techniques to quantify pollutant-protein and pollutant-DNA adduct relationships in blood and tissues;

- Develop better residue-effects links using long-term, mechanistic cause/effects studies;

- Modify rapid mutagenicity tests to improve their applicability to tissue extract assays.

The subgroup developed a number of general points relevant to all of the indicators discussed. They are as follows:

- In using measurement of residue concentrations as an indicator, it is vital to the proper interpretation of the results that it be linked to effects analysis (i.e., used in conjunction with other indicators);

- No single test is sufficient; a number of different indicators should be used in any given field study;

- Analysis must address the combined effects of major pollutants as this is what is found in the field;

- The mechanism of the effect must also be addressed. Without an understanding of mechanistic links, the indicators have little or no predictive value.

6. REPORT FROM THE SUBGROUP ON REPRODUCTION/DEVELOPMENT, PHYSIOLOGY, BEHAVIOR, AND POPULATION

After selecting a recorder (Dr. Judith Weis, Rutgers University) and a spokesperson (Dr. Joel O'Connor, NOAA), this subgroup listed possible finfish indicators of toxic pollution for each of the subcategories: reproduction and development; physiology; behavior; and population. Initially, the subgroup was asked to identify all possible indicators without discussing the worthiness of a particular choice. This resulted in a long list of indicators that was then consolidated by merging related and redundant indicators, and then deleting those considered least useful or outside the scope of the subgroup.

During this discussion, fishery closures (i.e., closure of a fishery by a regulatory agency due to contamination of the fish by toxic chemicals) were considered. The subgroup questioned whether closures should be considered as an indicator at all, and then agreed, with reservation, to consider them as a separate category.

The subgroup also decided to consider resistance or acclimation to pollutants when evaluating indicators. The subgroup agreed to consider and evaluate pollutant resistance as a separate category. Table 6 presents the final list of indicators considered for evaluation and ranking for each category.

The subgroup then tried to determine the relevance of each final indicator to each endpoint (fishes, ecosystem, and human health). The subgroup found that all of the indicators listed applied to the health of individual fish and fish populations and that none applied to ecosystem or human health effects.

After agreeing on a final list of finfish indicators, the group evaluated each indicator based on EPA criteria. Tables 7-10 summarize thesse findings. The presence of "unknown" indicates that the subgroup

TABLE 6. LIST OF INDICATORS CONSIDERED FOR RANKING BY THE SUBGROUP ON REPRODUCTION AND DEVELOPMENT/PHYSIOLOGY/BEHAVIOR/POPULATION

- Reproduction and Development
  - number of eggs
  - embryo viability
  - cytogenetics
  - larval development and viability
  - growth
  - fin regeneration
- Physiology
  - respiration
  - hematology
  - endocrinology
  - swimming stamina
- Behavior
  - schooling
  - avoidance/attraction
  - reproduction
  - predatory behavior
  - food habits
  - miscellaneous (predator avoidance/orientation/coordination/refuge-seeking behavior)
- Population
  - abundance
  - age structure profile
  - spatial distribution profile
- Fishery Closures
- Pollutant Resistance (acclimation)

TABLE 7. REPRODUCTION AND DEVELOPMENT

Criterion	Indicator					
	Number of Eggs	Embryo Viability	Cytogenetics	Larval Development	Growth	Fin Regeneration
Unequivocal Meaning	No	No	Yes	No	No	No
Cost-Effectiveness	Yes	Yes	Yes	Yes	Yes	Yes
Availability	Yes	Yes	Yes[1]	Yes[2]	Yes	Yes
Applicability						
-early/late sensitivity	Early	Early	Early	Early	Early	Early
-pollutant-specific	No	No	No	No	No	No
-species-specific	No	No	No	No	No	No
-scope-specific						
spatial	Unknown	Yes	Yes	Yes	Yes[3]	Yes
temporal	2-6 mo	days or weeks[4]	few days to 6 mo	1 week to 2 mo[5]	1 week to 2 mo[5]	1 mo to 1 yr

1 Needs expertise
2 But not widely
3 For resident species
4 Could be a function of the female taking up toxicants and passing them on to the embryo, in which case the time scale could be 2-6 months.
5 An extreme case would be up to a year

TABLE 8. PHYSIOLOGY

Criterion	Indicator[1]			
	Respiration	Hematology	Endocrinology	Swimming Stamina
Unequivocal Meaning	No	No[3]	No	No
Cost-Effectiveness	Yes	Yes	Unknown	Yes
Availability	Yes	Yes	Unknown	Yes
Applicability				
-early/late sensitivity	Early[2]	Early	Early	Late
-Pollutant-specific	No	No	No	No
-Species-specific	No	No	No	No
-Scope-specific				
spatial	Yes[2]	Yes[2]	Yes[2]	Yes
temporal	Yes[2]	No	Yes	No

1 There may be other potential indicators (e.g., scope for growth: the amount of energy available to an organism for growth and reproduction in excess of the energy required for maintenance)

2 Can be

3 Potential is there

TABLE 9. BEHAVIOR

Criterion	Indicator					
	Schooling	Avoidance/ Attraction	Reproduction	Predatory Behavior	Food Habits	Misc. Behaviors
Unequivocal Meaning	No	No	No	No	No	No
Cost-Effectiveness	No	Yes	No	No	Yes[2]	Yes[4]
Availability	Yes	Yes	Yes	Yes	Yes	Yes
Applicability						
-early/late sensitivity	Early	Early	Early	Early	Late	Early
-pollutant-specific	No	No	No	No	No	No
-species-specific	No	Yes	No	No	No	No
-scope-specific						
spatial	Yes	Yes	Yes	Yes	Yes[3]	Yes
temporal	Yes	Yes	Yes	Yes	Yes	Yes

1 Must be schooling species
2 Can be
3 Depends on species
4 Fairly cost-effective

TABLE 10. POPULATION

Criterion	Indicator		
	Abundance	Age Structure	Spatial Distribution
Unequivocal Meaning	No	No	No
Cost-Effectiveness	Yes[1]	Yes[1]	Yes
Availability	Yes	Yes	Yes
Applicability			
-early/late sensitivity	Late	Late	Early
-pollutant-specific	No	No	No
-species-specific	No	No	No
-scope-specific			
spatial	Yes[2]	Yes[2]	Yes
temporal	No	No	Yes

1 For local stocks
2 For anadromous and resident species

was unable to determine or agree upon the relationship between the indicator and the criterion. The two potential finfish indicators, pollutant resistance and fishery closures, are not directly related but are presented together in Table 11, for convenience.

Following evaluation, each indicator was ranked within its category on the first three criteria only (unequivocal meaning, cost effectiveness, and availability). The ranking within categories was performed using the four methods specified:

- o Select the best single indicator,
- o Select the best few indicators,
- o Give a numerical rank to each indicator, and
- o Classify each indicator as "good" or "bad."

Tables 12-15 summarize the results of the rankings. Although the indicators "pollutant resistance" and "fishery closures" were not ranked, the group agreed to present the evaluations of these indicators to the workshop. Although the group did not agree how to rank these two indicators, this does not imply that they are not useful indicators. In fact, these two indicators met the workshop's criteria well.

TABLE 11. POLLUTANT RESISTANCE AND FISHERY CLOSURE

Criterion	Indicator	
	Pollutant Resistance	Fishery Closures
Unequivocal Meaning	Yes	Yes
Cost - Effectiveness	No	Yes
Availability	Yes	Yes
Applicability		
-early/late sensitivity	Late	Late
-pollutant-specific	Yes	Yes
-species-specific	No	No
-scope-specific		
spatial	Yes	Yes[1]
temporal	No	Yes

[1] For resident species

TABLE 12. REPRODUCTION AND DEVELOPMENT

Indicator	#1	Best	Relative Rank	Good/Bad
Number of eggs			4	Good
Embryo viability		X	3	Good
Cytogenetics	X	X	1	Good
Larval development and viability		X	2	Good
Growth			6	Good
Fin regeneration			5	Good

TABLE 13. PHYSIOLOGY

Indicator	#1	Best	Relative Rank	Good/Bad
Respiration			3 or 4	Good
Hematology	X	X	1	Good
Endocrinology			3 or 4	Good
Swimming Stamina		X	2	Good

TABLE 14. BEHAVIOR

Indicator	#1	Best	Relative Rank	Good/Bad
Schooling		X	3	Good
Avoidance/ Attraction	X	X	1	Good
Reproduction			5	Bad
Predatory Behavior			6	Bad
Food Habits			4	Good
Miscellaneous Behaviors		X	2	Good

TABLE 15. POPULATION

Indicator	#1	Best	Relative Rank	Good/Bad
Abundance		X	2	Good
Age Structure Profile			3	Good
Spatial Distribution Profile	X	X	1	Good

7. SUMMARY REPORT FROM SUBGROUP ON IMMUNOLOGY

The field of immunology is contributing to new, sensitive, and rapid methods for the detection and identification of microorganisms in animals, humans, and the environment. These methods, such as enzyme-linked immunoassays, macrophage activity assays, and passive hemolytic assays, may be used to detect changes in animals caused by toxic contaminants. The Immunological Indicators subgroup considered which methods might be applied to detect changes in the immune system of finfish caused by contaminants.

The immune system in higher animals, including finfishes, is based on antigen exposure, resultant antibody or cellular response, and eventual protection (if a disease agent is involved). The subgroup approached this problem in the chronological order of the immune response: first considering the afferent immune response--pickup and processing of antigen, and then the efferent immune response--producing the physiological result, e.g., antibody, cellular activation, etc. Table 16 presents the inital list of immune indicators.

This subgroup assigned points to determine the relative rank of the monitoring methods relying on the immune response, with 1 the lowest rating, and 5 the highest rating. (See Table 17). Evaluations are summarized below.

MACROPHAGE TESTS

One method, which included examining a combination of macrophage phagocytosis, macrophage killing of microorganisms, and macrophage chemoluminescence, was judged a very good method. Two committee members considered this the best and the other two members considered it among the top three methods.

The members also considered the use of macrophage phagocytosis alone a good method, but not nearly as powerful as the combined

TABLE 16. INITIAL LISTING OF IMMUNE INDICATORS

- Macrophage indicators
  - phagocytosis
  - chemotaxis
  - pinocytosis (soluble particles)
  - killing
    - microbes
    - tumor cells
  - chemoluminescence (a technique)
  - aggregates of macrophages (in kidney, for example)
  - melanin accumulation by macrophages
  - MAF (macrophage activation factor)
  - MIF (macrophage inhibition factor)
- Lymphocyte indicators
  - T-lymphocytes
    - blastogenesis
    - cytotoxicity
    - lymphokine production
    - delayed type hypersensitivity
    - graft rejection (fish scale rejection)
  - B-lymphocytes
    - blastogenesis
    - antibody production (Jerne assays)

- Humoral antibody
  - Direct detection of antibody in serum to specific microbes via techniques of agglutination, precipitation, or ELISA

- Disease resistance
  - Directly measured by experimental animal exposure to a specific pathogen
  - Assessed epidemiologically; unknown field challenge

- Nonspecific defense mechanisms
  - CRP (C-reactive protein in blood)
  - natural killer cells
  - nonspecific antibody-like molecules
  - interferon

TABLE 17. RANKING OF METHODS SUITABLE FOR FIELD-TESTING AT PRESENT

Method	Usefulness, Unequivocal Meaning	Cost-Effectiveness	Availability
Macrophage tests			
phagocytosis alone	4	4	4
phagocytosis, chemoluminescence and killing	4.5	5	3
chemotaxis	3	5	4
melanin & aggregation	2	3	4
pinocytosis	4	4	4
T-lymphocyte grafting	3	3	2
B-lymphocyte Jerne assay	4	3	4
Humoral			
Antibody produced in injection	4	2	4
Antibody, non-specific in field	2	4	5
Disease Resistance			
Experimental exposure	3/1[b]	3	
Epidemiologically measured exposure	3/1[b]	3	5
Natural killer cells	2	3	2

[a] Rating scheme: 1 = lowest, 5 = highest
[b] Divided opinions

approach described above. Adding the other two macrophage indicators to a monitoring scheme was considered cost-effective. Other macrophage tests were considered as indicators, but ranked low in comparison to the above.

DISEASE RESISTANCE

The second highest ranked category of toxic indicators from immunological methods was the disease resistance area. The experimental measurement of disease resistance in feral fish (i.e., by injection of disease microorganisms) ranked high. However, a few members opposed this method on the grounds that its meaning is equivocal. The method also had a higher cost compared to others.

The group considered epidemiologic assessment of disease resistance to have one especially positive feature: it is universally available. Again, however, the opinion was divided as three members felt it was useful, and one member strongly opposed it.

JERNE ASSAY

A final method judged to be one of the strongest immunologic approaches was that of Jerne assay as applied to detect B-lymphocytes as antibody-producing cells. This method received strong support from most members and moderate support from all. Members also considered it widely available and cost-effective.

OTHER METHODS

Other methods considered at least moderately useful by a majority of the members were the following:

- Blood levels of antibody (humoral) in feral fish injected with microorganisms (not well tested, somewhat expensive, widely available);

- o Graft rejection (T-lymphocyte involvement), not well tried, not widely available, but promising.

The ranking of methods showed that all methods are generally applicable to assessing toxic contaminant impacts on finfish. Macrophage assessments were given high utility. These methods also can be pertinent to assessing impacts on the ecosystem as well as on human health, but are less direct measures of such impacts.

Recommended Immune Indicators for Field Testing as Monitoring Tools

Based on the evaluation criteria and ranking by the four suggested methods, the recommended ranking of immune indicators was as follows:

(1) A combined macrophage assay consisting of chemoluminescence, phagocytosis, and killing ability;

(2) Experimental measurement of disease resistance in feral fish by injection of known antigens or microorganisms;

(3) Jerne plaque assays, (B-lymphocyte measurement of antibody production);

(4) Blood levels of humoral antibody after defined injection.

Research Needs

The subgroup identified the following research needs for improving the usefulness of immune indicators of toxic contamination in finfish:

(1) Immune parameters for indicating toxic assessment should be derived from each section of the immunological system, including
   - o Macrophage assays,
   - o T-lymphocyte assays,

- o B-lymphocyte assays, and
- o Determination of disease resistance.

(2) Tests *in vivo* should be validated with assays *in vitro*, e.g., culture of immune cells or organs and demonstration of effects of additives.

(3) To obtain better statistical information on the immune assays, inbred lines of fish are needed.

(4) Because the endocrine system of finfish greatly affects their immune parameters, the effects of stress on the immune response in fish requires more investigation.

Workshop
on Finfish
as Indicators
of Toxic
Contamination

Sponsored by the
U.S. Environmental
Protection Agency,
Office of Marine and
Estuarine Protection

On behalf of the U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, I would like to invite you to participate in a workshop on Finfish as Indicators of Toxic Contamination in Estuaries. The workshop will be divided into various working groups of government and academic scientists and resource managers. The primary objective of the workshop is to determine preferred methodologies available for analyzing the significance of toxic impacts on estuarine finfish.

The workshop will be held at the Airlie House in Airlie, Virginia, on July 28-30, 1986. Technical Resources, Inc. (TRI) will provide technical and logistical support. Enclosed is a registration form. Please return this form to TRI by July 11, 1986.

Also enclosed for your information is a background document, Finfish as Indicators of Toxics in Estuaries, developed by Margaret McFadien-Carter of the University of Delaware.

I hope to see you in July and look forward to your contributions to this project.

Sincerely,

Tudor T. Davies
Director
Office of Marine and Estuarine Protection

enclosures

WORKSHOP ON FINFISH AS INDICATORS OF TOXIC CONTAMINATION IN ESTUARIES

Sponsored by the U.S. Environmental Protection Agency
Office of Marine and Estuarine Protection

July 28-30, 1986
Airlie House
Airlie, Virginia

Monday July 28

10:00 a.m. - 11:15 a.m.	Introduction: Tudor Davies Michelle Hiller Gary Petrazzuolo	Store Room
11:15 a.m. - 12:15 p.m.	Background Paper Discussion	Store Room
12:30 p.m. - 2:00 p.m.	Lunch	Main Dining Room
2:00 p.m. - 3:20 p.m.	Individual Subgroups	Livery, Silo House Hitching Post, Silo House Granary, Silo House West Room, Main House South Room, Main House
3:20 p.m. - 3:40 p.m.	Break	
3:40 p.m. - 6:00 p.m.	Individual Subgroups	Livery, Silo House Hitching Post, Silo House Granary, Silo House West Room, Main House South Room, Main House
6:00 p.m. - 7:00 p.m.	Reception - Cash Bar	Garden Room, Main House
7:00 p.m. - 8:00 p.m.	Dinner	Main Dining Room

Tuesday, July 29

7:30 a.m. - 8:30 a.m.	Breakfast	Main Dining Room
8:30 a.m. - 10:20 a.m.	Subgroup Summarizing	
10:20 a.m. - 10:40 a.m.	Break	
10:40 a.m. - 12:00 p.m.	Subgroup Plenary Session	Store Room
12:00 p.m. - 1:30 p.m.	Lunch	Main Dining Room
1:30 p.m. - 3:20 p.m.	Plenary Session	Store Room
3:20 p.m. - 3:40 p.m.	Break	
3:40 p.m. - 7:00 p.m.	Plenary Session	Store Room
7:00 p.m.	Cash Bar and Barbeque	The Lodge

Wednesday, July 30

7:30 a.m. - 8:30 a.m.	Breakfast	Main House
8:30 a.m. - 10:20 a.m.	Subgroup Summaries	Store Room
10:20 a.m. - 10:40 a.m.	Break	
10:40 a.m. - 12:00 n	Closing Summary	Store Room

FINFISH AS INDICATORS OF TOXIC CONTAMINATION IN ESTUARIES
July 28-30, 1986
Airlie House
Airlie, Virginia
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Background Paper
Finfish as Indicators of Toxics in Estuaries

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1. INTRODUCTION

1.1 Workshop Objectives

The primary objective of this workshop is to determine the relative usefulness of finfish indicators for toxic impacts in estuaries. Technical specialists and resource managers will help determine the most accurate, replicable, and cost effective methodologies immediately available for analyzing toxic impacts in estuarine finfish. Potential indicator methodologies will be evaluated based on workshop participants' answers to a set of questions and criteria provided by the workshop coordinators. These evaluations will offer estuary program managers a technical appraisal of the strengths and weaknesses of approaches using finfish indicators to assess toxic impacts.

1.2 Biotic Indicators for Toxic Pollution

Physical factors (e.g., hydrodynamics, sediment type, sediment transport) and chemical factors (eg., salinity, redox reactions, sorption/desorption processes, and chemical reactions) affect the fates and effects of toxic substances. Input rates of nonconservative pollutants to the environment can also influence their fate and the expression of toxic responses (Cairns, 1986a). Organisms can accumulate, and transport, metabolize (resulting in both detoxification and intoxification), and physically process (e.g., fecal pellet formation) toxic compounds, thus affecting the physical

and chemical factors that influence the fates of toxic substances in the environment. Consequently, the impact of toxics in estuaries depends on both abiotic and biotic characteristics and processes that interact through complex relationships. Pollutant loads and ambient concentrations may not, in themselves, offer good predictions of toxic impacts. Because of the variability of environmental conditions, measurements of effects of toxicity often provide a more meaningingful indication of environmental quality (Cairns, 1986b). Field analyses of toxic responses are desirable to evaluate actual impact on any given estuary. It is also particularly desirable to identify members of the biota that will offer accurate early warning systems of pollutant damage.

2. OVERVIEW OF FINFISH AS INDICATORS OF TOXIC POLLUTION

2.1 Working Definitions of Toxic Pollution and Stress

The words "toxicants" and "toxics" will be used in this paper to mean chemicals that produce acute or chronic deleterious effects on finfish and/or other estuarine biota. Effects caused by toxics may be systemic, teratogenic and carcinogenic as well as any other effects that reduce reproductive fitness or shorten the individual's natural life.

The term "stress" may refer to any pressure or forcing principal that alters the natural processes (physiological, develop-

mental, or behavioral) of an organism. Stress induced by exposure to a toxic chemical could ultimately have negative or neutral effects.

Examples of negative stress would be toxic effects that ultimately shorten the organism's life, impair its reproductive success, or result in adverse, toxic effects in organisms higher on the food chain, through accumulation or metabolic transformations of toxic substances.

An example of stress with neutral effects might be the metabolism of a small amount of a toxic chemical to a non-toxic excretable daughter chemical, with no production of toxic, reactive intermediates. This type of stress utilizes energy reserves of the organism. However, the organism is not permanently impaired, and significant effects on reproductive success are unlikely.

Beneficial results of stress are feasible. An example here would be the stimulation of metal binding proteins by exposure to a toxic metal that increases an organism's resistance to subsequent exposures to toxic metals. However, these benefits generally apply only to further toxic stresses, and often do not include larger ecosystem effects.

For the remainder of this paper both neutral and negative effects will be considered. The emphasis, however, will be on the

concept of negative stress. In summary the following classifications of "stress caused by toxic chemicals" will be considered:

Neutral stress: pressure from toxic chemicals on finfish causing altered physiology or morphology without permanent impairment of the organism's lifespan or reproductive success.

Negative stress: toxic pressures causing acute or chronic and subiethal effects that decrease an organism's lifespan and/or its reproductive success. These pressures might result in reduced survival of offspring, altered fecundity, lowered growth rate, suppression of immune responses, pathological tissue changes, genetic changes, or anatomical or physiological changes deleterious to the individual organism.

Types of effects due to toxic stress considered in this paper are shown in Table 1. It is useful to distinguish among (a) pathological, (b) physiological without pathological, (c) pathologically-induced behavioral, and (d) strictly behavioral effects of toxic stress. The selection of stress indicators for this paper was based, in part, on identification by investigators of probable causative factors for the effects being studied. These are also shown in Table 1. While negative effects due to negative stresses are emphasized, neutral stresses and their effects are also considered.

Table 1
Survey of Effects of Toxic Chemicals in Finfish

I. Negative effects/negative stress: chronic, sublethal effects deleterious to individual organism.

Effect	Type of Effect	Documented Causative Agents
A. Bioaccumulaton/ Tissue Concentrations	Physiological/ pathological	PAH, PCB, Chlorinated hydrocarbons
B. Histopathological 1. Fin erosion, ulcers, cataracts 2. Neoplasms	Pathological	PAH, PCB, Chlorinated hydrocarbons
C. Immune effects (repression/stimulation)	Physiological: can lead to pathological	PAH, PCB, Metals
D. Reproductive and developmental effects	Physiological: can lead to pathological	Metals, PAH, PCB, Chlorinated hydrocarbons
E. Deleterious results reflected by enzyme alteration	Physiological	PCB, PAH, Metal
F. Deleterious metabolism of chemicals (toxification)	Physiological reflects pathological	PAH, PCB
G. General physiological alterations (gill respiration, osmoregulation)	Physiological/ pathological	PAH, PCB, Chlorinated hydrocarbons
H. Behavioral and population alterations due to effects on individuals	Pathologically or Physiologically induced behavior	PAH, Metals, Chlorinated hydrocarbons

II. Neutral effects/neutral stress: chronic effects with latered physiology but with no permanent impairment of organism.

Effect	Type of Effect	Documented Stress
A. Non-injurious enzymatic alterations	Physiological/ non-pathological	PAH, PCB, Metals
B. Metabolism of toxic parent to non-toxic daughter chemical (detoxification)	Physiological/ non-pathological	PAH, PCB

2.2 Historical Sources of Toxic Substances

The sources, fates and possible effects of toxics in estuaries currently are subjects of considerable concern among scientists and environmental managers. Toxics include polyaromatic hydrocarbons (PAHs) and other petroleum-related compounds, polychlorinated biphenyls (PCBs), pesticides, other synthetic organics, and toxic metals.

Petroleum-related sources include marine transportation, accidental spills, municipal wastewater discharges, refinery wastes, industrial wastes and urban runoff. Nonpoint sources such as urban runoff may constitute a major portion of petroleum pollution. There is also concern that atmospheric transport may contribute more to petroleum pollution of surface waters than previously believed or documented (National Research Council, 1985).

Primary sources of PCBs to the environment include leakage from closed electrical systems, such as transformers and capacitors, and losses during the manufacture and use of hydraulic fluids, lubricants and heat transfer fluids. Other sources include adhesives, plasticizers, pesticide extenders, and dyes. PCBs disposed in landfills also may represent a significant pollution hazard.

Pesticides typically reach surface waters from nonpoint sources, runoff and atmospheric settling which result from agricul-

tural, parkland, suburban, or urban pest control efforts. Another source of increasing concern is municipal wastewater effluents, which contain surprisingly high pesticide concentrations as a result of household use (Carter, 1985).

Other synthetic organic toxicants of particular concern include: dioxins, phthalate esters, haloethers, chlorinated hydrocarbons (other than pesticides), organometallic compounds, nitrobenzenes, nitrosamines, benzidines, phenols, acrolein, acrylonitrile, dichloro-5-fluoromethane, benzo(a)pyrene. These chemicals are released from a number of industrial, domestic, and agricultural sources.

Toxic metals include: aluminum, antimony, arsenic, barium, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, gold, iron, lead, lithium, manganese, mercury, molybdenum, nickel. palladium, platinum, selenium, silicon, silver, tellurium, thallium, tin, titanium, vanadium, zinc and zirconium. Sources of most of these metals are primarily industrial. A partial list of industrial metals considered toxic to humans is demonstrated in Table 2.

Table 2
Abbreviated Listing of Industrial Uses of Some Potentially Toxic Metals

* = Considered highly toxic
Table developed from data available in Berman (1980)

Metal	Modern Use
*Antimony:	alloyed with lead, tin and copper, a flame retardant in paints, enamels and lacquers: also used in printed type.
*Arsenic:	smelting, rodenticides, insecticides, herbicides, glass and enamel manufacture.
*Barium:	electroplating, glass manufacturing, sugar refining; also in television tubes and explosives.
Beryllium:	alloyed with copper in electrical equipment; used in producing optical glass, nuclear reactors.
Bismuth:	used in electrical fuses, facial powders and producing artificial pearls.
*Cadmium:	used in electroplating, engraving, dental amalgam, glass manufacture, ceramic glazes; Jaune brilliant (Cadmium sulfide) used to color glass, soaps, fireworks and textiles.
Chromium:	used in manufacture of stainless steel, in photography and as corrosion inhibitor.
Cobalt:	used in alloys and nuclear technology.
*Copper:	insecticides, fungicides, germicides; as pigment in textiles and ceramics.
Gold:	other than currency, used medicinally, in printed circuits, semi-conductors and in the space industry in glass to metal seals.
Iron:	other than manufacture of steel; ferric chromate in pigments, ferric hydroxide in water purification, the oxide as a polishing agent and pigment.
*Lead:	although reduced in paints, still high levels in putty and plaster, glazed earthenware, in pewter, chafing dish candles, hair dyes, color in magazines.

Table 2
Abbreviated Listing of Industrial Uses of Some
Potentially Toxic Metals

Metal	Modern Use
*Lithium:	aerospace alloys, lubricating greases, metal cleaners photography.
*Molybdenum:	as alloy for special steel in rifle barrels, propeller shafts, boiler plate, x-ray tubes; as lubricant additive (toxicity has been demonstrated to be species specific).
Nickel:	storage batteries, spark plugs, cooking utensils, detergents; Raney nickel (equal parts aluminum and nickel) used for hydrogenation of oils.
Palladium:	metal plating; catalyst in electrical industry; as alloy with Ag, Au or Cu for jewelry and dentistry.
Platinum:	dentistry, jewelry, and in electrical industry.
*Selenium:	manufacture of plastics, rubber, ceramics, ink, glass, paint pigments, photoelectric cells.
*Silver:	besides tableware, jewelry, and dentistry, as alloy with many metals. Also as steel coating; in manufacture of solder.
*Thallium:	amalgam with Hg; alloy in switches; in manufacture of pigments and dyes, as rat poison.
Tin:	diverse uses: food containers, electrical, radio, automobile parts; color for china, fabric dying; organic complexes as biocides.
Titanium:	for strengthening steel; useful alloy with many metals; titanium dioxide used in creams, powders, sun protection, in paint, plastics and leather work, trichloride in laundering.
Vanadium:	allows with Pb, Mn, Cr:: rust resistance, strengthening steel, photographic developer, dying cottons, silks, leathers and furs.
Zinc:	manufacture of bronze and brass. coating on iron, steel.
Zirconium:	shielding in nuclear submarines and power reactors, used in production of paints, flashbulbs and detonators; chloride and acetate as textile water repellents.

2.3 Use of Finfish as Indicators of Toxic Pollution

Criteria for evaluating toxic effects in ecosystems have been suggested by the National Academy of Sciences (NRC, 1981). They surveyed laboratory and field methods, including chemical characterizations, single-species tests, multi-species tests, and ecosystem tests. Ultimately they suggested that:

> "research should be conducted to develop test procedures that can provide multiple sets of data. Tests should be designed to provide short-term results about long-term effects."

This NRC study recommended that four classes of information should be collected:

- Characterization of test substance
- Physiological responses of species
- Multi-species responses
- Ecosystem responses.

Collecting all four classes of data will serve the following purposes for testing toxic effects:

- determine partition coefficients for movement of chemicals in the system,
- identify the toxic potential for major transformation or degradation products,
- account for variability in natural systems affecting dose to biota or exposure time within a compartment, and help distinguish natural variations from chemically induced variations in ecosystems (NRC 1981).

By evaluating physiological responses of sensitive species in the laboratory, discrete morphological genetic, biochemical, and pathological effects of particular chemicals can be identified. Subsequent ecosystem response studies can evaluate the extent to which these processes are expressed in the field, and the ways in which occurrence of morphological, genetic, biochemical and pathological changes in single species in situ affect the abundance and distribution of the species of concern and those that interact with them. Identification of some single species impacts in the field may then offer pollution abatement monitoring programs rapid identification methods for long-term problems.

Finfish, as a group, offer monitoring programs several benefits. Analyses of physiological and pathological effects of parent and daughter chemicals have demonstrated that finfish are susceptible to a number of readily identifiable and some less well understood effects of toxic stress (Couch and Harshbarger, 1985; O'Connor et al., 1986; Malins et al., 1980, 1983, 1985; Murchelano and Wolke, 1985; NRC 1985; Hargis et al., 1984). They are also capable of transforming parent chemicals into more toxic daughter chemicals (TetraTech, 1985). They are at sufficiently high trophic levels that they are likely to serve as early-warning indicators because of bioconcentration effects. Altered finfish physiology and pathology in field specimens therefore seem likely to offer environmental managers particularly useful warning systems for chemical pollution of estuaries.

One potential problem with the use of finfish as indicator organisms is their mobility. The benefits and disadvantages of using relatively mobile organisms as toxic stress indicators will be discussed later in this background paper.

2.4 The Problem of Background Noise

To identify pollution due to toxics, it is necessary to distinguish between toxic effects and background noise in any given population or ecosystem. Background noise may be defined as natural variations in measurable biotic processes that are not causally related to the toxic pollutant or other disturbance that is of concern. Differentiation between toxic effects and background noise can be difficult to accomplish. Clear causal relationships should be established in the laboratory whenever possible. It is necessary to measure background noise in all monitoring programs to obtain valid stress analyses in the field. Robert Livingston has provided an excellent review of the problem of evaluating background noise; this review is included as Appendix A of this document.

3. FINFISH INDICATORS OF TOXIC POLLUTION

The remainder of this background document describes specific effects of toxics on finfish as listed in Table 1. An introductory chapter summarizing and characterizing the effects reviewed is followed by a series of papers by specialists describing methodologies for analyzing particular effects. In some cases, where a previously published review article or technical report presents the necessary information on a given method or methods, that article or report has been included with the author's permission in lieu of preparing original text for this report.

Effects are discussed under the following major headings: Bioaccumulation/Tissue concentration; Histopathology: Non-oncogenic; Histopathology: Oncogenic; Immune Effects; Effects on Reproduction and Development; Enzyme Alterations; Metabolism of Toxicants; General Physiological Alterations; and Population Alterations. Many effects fall into more than one of these categories, and it is not intended that great significance be placed on the details of the categorization presented here for purposes of discussion. The objective of the workshop discussions will be to address strengths and weaknesses of individual measurable effects as indicators, and the merits of methodologies used for these measurements. Each indicator will be evaluated on its own merits, so that assignment to one or another general category should not affect the evaluation.

3.1 Bioaccumulation/Tissue Concentrations

The biological uptake of toxics through food, water, sediment contact, or a combination of exposures and retention in tissues is called bioaccumulation. A number of factors affect this process. The bioaccumulation potential of a substance is dependent on its chemical properties, the affected organism's mechanisms for uptake and elimination, and the environmental factors influencing its bioavailability. Examples of the variability of intrinsic and extrinsic chemical processes in bioaccumulation of various toxicants are given by trace metals. While bioaccumulation of Cd and Cu are primarily dependent on free ion activity, bioaccumulation and toxicity of silver appears dependent on formation of chlorocomplexes (Engel et al., 1981).

Physical and chemical partitioning of toxics as they enter an estuary influences exposure routes to the biota. Benthic fauna and demersal fish are most likely to be affected by toxicants associated with particulates. It has been demonstrated that direct sediment contact contributes significantly to PCB bioaccumulation in demersal fish. Although dietary contributions of PCBs were high, fish without direct sediment contact accumulated significantly lower PCB residues (Rubenstein et al., 1984).

Bioaccumulation is ultimately determined by the organism's ability to metabolize, transform, and excrete a toxicant. Clearance ability can be related to amounts of adipose tissue as well as to length or pattern of exposure to the chemical. Lipid binding due to hydrophobic interactions, has been demonstrated by the greater bioaccumulation of aromatic hydrocarbons over alkenes in petroleum polluted waters (Neff, 1976). Ultimate clearance rates appear to vary considerably among taxonomic groups of fishes and may even be species-specific (Neff, 1976; TetraTech, 1985).

Other biological influences on ultimate tissue burden include membrane permeability and the potential for translocation of the chemical from the absorption site to other tissues (TetraTech, 1985). For a more detailed discussion of this topic the reader is referred to O'Connor's review in Appendix B. Finally, it should be noted that low tissue burdens do not necessarily indicate insignificant effects of bioaccumulation since some metabolized daughter compounds are toxic in extremely small amounts (TetraTech, 1985).

3.2 Histopathology

3.2.1 Non-Oncogenic

Sublethal chronic exposure to certain toxics can cause cellular damage in the skin, liver, eye lens and intestines of

pelagic and benthic fish (Hargis et al., 1984; Hawkes, 1979). Chronic exposure to petroleum causes not only direct tissue lesions but also apparently via the lesions increases susceptibility to parasitism, bacterial infection and viral disease indirectly via these toxicant-induced lesions (Hodgkins et al., 1977; Sindermann, 1979).

Synergistic actions of toxicants can increase severity of tissue lesions. Hawkes (1979) demonstrated that while chronic exposure to PCBs alone caused sloughing of intestinal mucosa, the severity of the problem increased when the fish were exposed to combined PCBs and petroleum fractions. Fin rot and cataracts have been correlated with sublethal chronic exposure of finfish to sewage effluents, and synergistic actions of pollutants including toxics have been identified as a possible cause of these pathological disturbances (Hillman et al., 1986).

Controlled studies have specifically correlated integumental lesions with certain toxics. Finfish exposed to sediment contaminated with PAHs demonstrated severe lesions and ulceration within 8 days while control fish demonstrated no lesions (Hargis et al., 1984). Weis and coworkers have suggested that fin deterioration is a natural occurrence due to abrasion in demersal fish and that lesions result from inhibition of regeneration due to toxics (pers. comm. J. Weis, May, 1986). Methylmercuric chloride and cadmium chloride have been shown to retard fin regeneration in

fishes (Weis and Weis, 1978). Fin regeneration was also retarded by DDT, malathion, carboryl, zinc, parathion and PCBs. It was not strongly affected by quantitative diet changes or fish density pressures (pers. comm. J. Weis, May, 1986).

The possibility of adaptation to pollution is suggested by increased methylmercuric tolerance in female killifish correlated with increased fin ray count (Weis et al., 1981; Weis and Weis, 1984). A paper in preparation by J. Weis, P. Weis and Zimmerer discusses fin regeneration and its usefulness as a monitoring tool for toxics. The correlation of exposure to toxics, especially PAHs and PCBs, with integumental lesions including finrot, gill deterioration, and cataracts has been well documented and is reviewed more thoroughly, with descriptions of current methodologies for measuring the effects in Appendix C.

3.2.2 Oncogenic

Occurrences well above background levels of liver neoplasms in finfish in polluted estuaries have been clearly demonstrated over the last decade (Malins et al., 1980,1983; McCain et al., 1977, 1982). Such neoplasms have also been reported in fish from polluted fresh waters. While most estuarine and freshwater fish studies have concentrated on the effects of PAHs and PCBs there is evidence that heavy metals can also be correlated with carcinomas (Couch & Harshbarger, 1985).

Malins et al. (1985) have recently demonstrated field correlations of increased hepatic lesions among English sole with sediment contamination by aromatic hydrocarbons. In this case the dietary uptake of the chemicals was documented. Baumann et al. (1982) have similarly documented hepatomas in wild populations of English sole and tomcod.

Contradictory evidence has been presented with regard to cutaneous papillomas in demersal fish. While Dawe and Harshbarger (1975) demonstrated increased occurrence of these disorders in industrialized areas, Iwaoka et al. (1979) presented inconclusive data on the relationship of toxic effects and such superficial tumors.

Pituitary alterations can also be indicators of toxic stress. Pseudocysts developed in sheepshead minnows' pituitary glands when the fish were subjected to the herbicide trifluralin. These fish were consequently functionally damaged, demonstrating bone disorders including vertebral dysplasia (Couch, 1984).

In overview, there is a considerable body of literature indicating correlation of PAHs, PCBs, and heavy metals with increased occurrence of cutaneous carcinomas, liver neoplasms and histopathological changes in freshwater and estuarine finfish (O'Connor et al., 1986; and Malins, 1983).

The body of information showing correlations of toxic chemicals with lesions and subsequent infection and with carcinomas has led to considerable effort towards classifying tumors and monitoring finfish histopathology in polluted estuaries (Couch and Harshbarger, 1985; O'Connor et al., 1986; Whipple, 1984; Whipple et al., 1984). A detailed review of carcinomas is provided in Appendix D. An important objective of the present workshop will be to discuss strengths and weaknesses of available field monitoring methodologies for these effects, as well as what is known of the significance of the effects on individual organisms, populations and biological communities.

3.3 Immunology

As noted above, chronic sublethal exposure to toxic chemicals is believed to predispose finfish to parasitic, bacterial, fungal and viral infections (Hawkes, 1979; Weeks et al., 1986a; Whipple, 1984; Whipple et al., 1984)).

Investigations comparing estuarine species from polluted waters with controls from unpolluted areas have demonstrated that macrophage activity in finfish is markedly affected by water (or sediment) quality. Macrophages serve as the first line of defense against infection by uptake or endocytosis of disease agents. Chemotaxis (response to chemical stimulus), phagocytosis (uptake of particulates) and pinocytosis (uptake of fluids) are processes

involved in normal endocytosis. Research has demonstrated that each of these processes can be altered by the presence of environmental pollutants, increasing finfish susceptibility to infection and disease. However, variations are species-specific. Also, subsequent return of fish to clean water has been found to reverse macrophage activities to normal (Weeks et al., 1984, 1986a, 1986b).

Freshwater studies of the cellular immune response in finfish have further suggested that primary and secondary responses are diet related (Blazer et al., 1984). This would suggest the importance of toxicant uptake through ingestion.

Other studies have found that fish exposed to enderin had increased serum cortisol concentrations, contributing to the repression of tne immune response (Bennett et al., 1985 a,b). lt has also been suggested that analyses of pigmented macrophages from finfish reticulo-endothelial systems (RES) may serve as a means of monitoring immune alterations and fish health (Wolke et al., 1980).

A detailed review of immune effects of toxic chemicals including a description of state-of-the-art methodologies has been prepared for this report by Weeks, et al. (Appendix E)

3.4 Reproduction and Development

A number of studies have described reduced reproductive capacity, fecundity and gamete viability in fish exposed to chronic sublethal toxic stress (e.g. Engle, 1979; Whipple, 1984; Spies 1985). Surviving juveniles are often subject to abnormal hematopoesis (Longwell et al., 1983; Perry et al., 1984) as well as neurological and skeletal abnormalities (Weis & Weis, 1979). Some examples of effects of toxicants are discussed below. Detail on these and discussion of other effects of toxic chemicals on reproduction and development may be furthered during the workshop.

Monocyclic aromatic hydrocarbons (MAH), zinc, DDT, PCBs and total residual chlorine have frequently been correlated with reproductive and larval abnormalities. Increased petrochemical concentrations have been found correlated with egg resorption and abnormal reproduction. Benzene has been found to cause a particularly large number of effects including induction of egg resorption and association with gill parasites in adults. Blood cell destruction and decreased serum proteins in juveniles are also associated with benzene. When combined with zinc, benzene affects striped bass by severely accelerating parasitism, blood cell deterioration and a decrease in serum proteins.

Abnormal egg development has been directly associated with levels of DDT in striped bass ovaries (Whipple et al., 1984). DDT

is also associated with neurological defects in sheepshead minnow development (Weis & Weis, 1979). PCBs caused delayed egg maturation in striped bass (Whipple et al., 1984). Arclor 1254 (a PCB) likewise reduced survival of embryos and fry of sheepshead minnows (Hansen et al., 1973). Tests of toxicity of the cupric ion on eggs of spot and Atlantic silverside found considerable differences in sensitivity at time of hatching, with the silverside more severely affected (Engel et al., 1979). Cadmium, copper, nickel and zinc have all been associated with reduced egg viability in the striped bass (whipple et al., 1984). Exposure to a number of heavy metals caused skeletal defects in developing killifish juveniles. Mercury salts most severely affected skeletal development while lead impairs uncurling in certain estuarine fish after hatching (Weis & Weis (1979). Kepone was found to cause scoliosis, neurological impairment and impaired growth in juvenile sheepshead minnows (Hansen et al., 1977).

Killifish develop cyclopia and other optic abnormalities in response to a number of toxic chemicals. When exposed to carbaryl and parathion, killifish showed developmental arrest and cardiovascular abnormalities.

Longwell has shown that the chorion of certain fishes can become contaminated by oil-derived hydrocarbons and has suggested that species differences in these contaminations may be related either to species spawning habits with regard to depth of water

column or to the particular developmental stage at which eggs are exposed to the oil (Longwell, 1978). She has also shown significant correlations between surface-layer toxics and cytologic, cytogenetic and embryological health of mackerel eggs (Longwell & Hughs, 1980). By analyzing the yolk sac membrane in fish eggs, Longwell and Hughs (1981 a,b) have demonstrated significant correlations between mitotic-chromosome irregularities and contamination with hydrocarbons and heavy metals. This and subsequent studies have focused on decreased egg health, defined by Longwell and Hughes as "embryo moribundity, chromosome-mitotic abnormalities , development rate, cell differentiation problems, gross embryo malformation and total egg number sampled." Their studies show that poor egg health is related to toxics, salinity and temperature (Longwell and Hughes, 1980, 1982a,b; Longwell et al., 1984; Chang and Longwell, 1984, 1985). Longwell discusses methodologies and results of much of this work conducted on fish of the New York Bight in Appendix F.

In summary, effects of toxic stress on reproduction and development of estuarine finfish are numerous but variable according to type of toxicant and type of fish. A manual prepared by Whipple et al., 1984b provides methodology for the analyses of each aspect of reproductive health of striped bass. An account of congenital effects of toxics on a range of estuarine fish can be found in Weis and Weis (Appendix G) while Spies discusses reproductive impairment/success related to toxic stress in Appendix H.

3.5 Enzymology

3.5.1 Enzyme Induction

There is a considerable body of evidence documenting the effects of chronic sublethal exposure to several types of toxics on the mixed-function oxygenase system (MFO) in finfish.

The MFO system consists of an electron transport system that oxidatively transforms nonpolar lipophilic organic compounds into more water soluble metabolites. In fish it has been located primarily in the liver, with lesser activity occurring in the kidney, gills, gonads and heart. It consists of NADPH/cytochrome P-450, reductase, cytochrome P-450 and phospholipids, which combine with NADPH, oxygen and a substrate. The substrate may be transformed into highly reactive toxic intermediates, and to more or less toxic daughter compounds prior to excretion. This has lead some investigators to refer to the MFO system as a toxification /detoxification system (Neff, 1984). The activity of the hepatic MFO system is induced (increased) in finfish exposed to petroleum and PAHs (Lech et al., 1982; Neff, 1976; Neff 1984; Stegeman, 1981). PCBs, dioxins and heavy metals may also induce MFO activity (Lech et al., 1982; NRC, 1985). Natural environmental factors and intrinsic biological chemicals (e.g. testosterone) may also increase or decrease MFO activity, suggesting that it be used with caution as an indicator of environmental pollution (Neff, 1984; NRC, 1985).

However, induced MFO activity can indicate rather specific exposure to several organic pollutants (Neff, 1984). This fact combined with its potential to increase the toxic load in the organism through production of carcinogenic metabolites encourages continued research on the system. Stegeman has provided a review of toxic effects on enzyme systems, particularly the MFO systems in Appendix I.

The components and function of the MFO system, sometimes referred to as the toxification/detoxification system, were introduced above. MFO enzymes metabolize environmental xenobiotics and endogenous steroids (Spies et al., 1982). The resulting molecules (e.g. sulfates) are small and polar, thus easily excreted. While this prevents accumulation of PAHs and certain other toxics in tissues, their oxidation can produce carcinogenic and mutagenic daughter compounds (Kurelec et al., 1977; Spies, 1982; Varans et al., 1980). The system responds in a similar fashion to PCBs (Gruger et al., 1977, Spies et al., 1982).

Ingested PCBs and PAHs increase aryl hydrocarbon hydroxylase (AHH) and microsomal proteins in some flatfish (Spies, 1982). As a result of these studies, it has been the suggested that activity of the MFO system be used as an indicator of at least petroleum pollution (Payne and Penrose, 1975; Stegeman, 1978,1980). Toward this end research has demonstrated that, unlike mammalian systems where there are specific inducers, the finfish show similar

qualitative responses to petroleum, PCBs and a range of other xenobiotics (Spies et al., 1982). However, DDT and DDE do not induce MFO activity in flatfish (Addison et al., 1977; Spies, 1982), but do induce MFO activity in white croakers (Brown et al., 1982). Thus MFO induction by chlorinated hydrocarbons may not be universal in finfish.

While PCBs from sewage effluents seem poorly metabolized by flatfish, chronic sublethal exposure to PCBs seems to hinder reproductive success in starry flounder. This suggests a possible indirect toxic effect of PCB metabolic intermediates via the MFO system (see Spies's Appendix H).

Metallothioneins are components of an intracellular mechanism for sequestering and detoxifying trace metals. Metallothioneins are metal binding, low molecular weight, cysteine rich proteins that lack aromatic amino acids. Metallothionein systems have been studied in fish and other animals, including mammals. While the metallothionein system is believed to sequester metals that might bind to sensitive cellular sites, it has also been found to be part of the normal metabolism of copper and zinc in mammals (England Rouseyidi pers. comm.; Jenkins et al., 1982). Metallothionein synthesis is induced by low levels of a number of metals resulting in highly stable metal-thiol bonds which allow the organism to tolerate increasing amounts of the trace metals. Metallothioneins thus serve as an excellent specific indicator for metals, but their activity has caused difficulties in analyses of potential

toxicity of these metals. Cytosolic metal distribution studies offer a possible approach to resolving such difficulties, as well as a method for clarifying avenues of metal uptake and synergistic activities of metals with other pollutants.

A survey of the research on metallothionein systems in particular and current methods for assessing toxicant metabolic activities in general can be found in Jenkins' review in Appendix J.

3.5.2 Molecular Pathology

Blood enzymes have long been used for clinical diagnosis in mammals. Changes in concentrations of tissue-specific enzymes are routinely used in the diagnosis of liver and heart disease or damage, for instance. Activity in fish tissue-specific enzymes may also be useful for diagnosing pollution caused cellular damage and pathological conditions. For example, delta amino levulinic acid dehydratase (ALA-D) is an enzyme involved in the synthesis of hemoglobin and other porphyrin-proteins. Lead inhibits ALA-D activity in finfish erythrocytes (Beritić et al., 1977).

Two other blood enzymes of use in analyzing pollutant damage to fish are glutamate oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT). Activity changes in these enzymes primarily reflect liver damage although GOT can also reflect damage to heart tissue. GOT activity decreases with

exposure of certain finfish to lead but increases with exposure to phenol, PCBs and municipal sewage effluent with synthetic organics (Neff 1984). GPT increases with exposure to all of these pollutants (lead, phenol, PCBs, municipal sewage effluent). Otner blood enzymes found to vary in activity when fish are exposed to pollutants are LDH (lactate dehydrogenase), alkaline phosphatase, glucuronidase, amino levulinic acid dehydratase and isocytric dehydrogenase. However, several of these enzymes, as well as creatine phosphokinase, show less change in the plasma with CCl_4 exposure than do GOT and GPT (Neff, 1984). At present then, ALA-D, GPT, and GOT appear the most promising blood enzyme indicators of pollutant mediated damage. Further investigations of pollutant effects on enzyme systems, and of non pollutant effects on blood enzyme activity may expand this list.

Tissue enzyme analyses have included in vitro assays and in vivo studies. The results of the two forms of analysis are often quite different. Also, it is not always clear whether pollutant mediated enzyme changes will result in significant changes in metabolic processes in the fish (Neff, 1980).

Relationships have been demonstrated between toxicant exposures and altered activity of some tissue enzymes. The gill epithelia contain several adenosine triphospatases (ATPases). These are important in osmotic and ionic regulation. Direct correlations between chronic sublethal exposure to pollutants and

changes in activity of these enzymes have been documented (Miller and Kinter, 1977; Neff, 1984). Likewise altered acetylcholinesterase (AChE) activity in the brain, gill and muscles of fish clearly correlates with exposure to chlorinated hydrocarbons, carbamate and organophosphates (Neff, 1984).

As with blood enzymes, further research would be needed to determine what relationships exist between tissue enzyme activities in finfish and most toxicant stresses, and between any altered enzyme activities and ultimate biological effects.

3.6 Physiology

Alterations of physiological functions such as osmoregulation and respiration can also indicate toxic stress in fish. However, distinguishing physiological effects from other effects is sometimes difficult. Coho salmon smolt demonstrate osmoregulatory failure under toxic burdens, for example, but it has been suggested that this failure is secondary and symptomatic of other negative stresses. The investigators found no evidence of toluene or naphthalene altering osmoregulatory ability in the smolt as a function of salinity (Stickle et al., 1982).

While respiratory rates have been examined as indicators of sublethal stress by toxics (esp. petroleum), results are again

inconsistent because of the numerous other factors that affect respiration. It has also been shown that responses vary considerably among animal classes. Oxygen-consumption rates are depressed in silver stressed cunners and mud snails, but elevated in all bivalves studied (Goulde et al., 1977). Severely ulcerated gills of finfish caused by PAH exposure do clearly demonstrate impaired respiration (R. Huggett pers. comm., April 1986). A potential answer to the problems of separating individual physiological effects from other possible primary causes is the use of an energy budget to evaluate toxic stress effects on respiration and other physiological parameters (Bayne et al., 1976,, 1979). Such a method is valuable because it avoids a need to separate intrinsic and extrinsic effects on respiration and offers comparisons within and among species on integrative stress indices such as growth rate (NRC, 1985).

Bioenergetics methods have been effectively used as toxic stress indicators by several investigators (Gilfillan et al., 1985; NRC, 1985). Estimations of the catabolic energy of proteins and amino acids, for example, can be determined by determining the ratio of oxygen used to nitrogen excreted (O:N ratio).

Another way of assessing physiological effects of pollutants is through correlation of altered enzyme activity, as discussed above, with altered physiological function. ATPases may be either stimulated or inhibited by pollutants including chlorinated hydro-

carbons, metals and crude oil (Poston et al., 1979; Lorz et al., 1978; Miller et al., 1977). These ATPases are found in finfish gills and control ionic and osmotic regulation.

Other biochemical changes induced by toxicants have also been shown to correlate with specific physiological dysfunctions and toxic stress. Changes in the adenylate energy charge (AEC) for instance, reflect the metabolic energy available from the adenine nucleotide pool. Stresses altering this pool then alter available metabolic energy (Neff, 1984). Declines in liver glycogen have been shown to coincide with hyperglycemia (Neff, 1984). Decreased growth rates and fecundity as well as altered energy metabolisms have been correlated with toxicant induced depression in liver glycogen and hypoglycemia (Conan, 1982; Neff, 1984).

Thus physiological dysfunctions may serve as relatively easily observed symptoms of some biochemical and enzyme impairments due to toxic stress.

3.7 Population

Population effects can include those caused by changed reproductive rates, or changed distribution and migration patterns. Effects of suppressed reproductive rates on population density have been clearly demonstrated in the diminishing population of the striped bass in San Francisco Bay. The suppressed reproductive

rates have been shown by these authors to correlate with toxic concentrations in the Bay (Whipple et al., 1984).

Several laboratory studies have demonstrated that certain fish can avoid toxics including DDT, enderin, and Duroban. There is, however, no proof that fish avoid toxicants in the field (Hansen et al., 1972, 1974; Hansen, 1969). However, if they do, the potential for beneficial effect on natural populations is considerable. If the behavior is genetically controlled, it would tend to increase through selection in environments with "hot spots" of pollution. The hotspots would be increasingly avoided by such species and the population would suffer less exposure to toxic effects.

Observations of the entire life cycle of individuals under toxic stress can serve as a first level of research in effects of toxics on whole populations. Such laboratory studies have suggested severe effects on reproductive efficiency in sheepshead minnows (Hansen and Parrish, 1977; Hansen et al., 1977).

One of the greatest problems in analyzing effects of toxics on populations is separating direct toxic effects from natural or non-pollution related variations including overfishing. Appendix K provides a discussion of this problem.

SUMMARY

The effects of toxic stress on finfish may be deleterious as in the case of neoplasms or neutral as in the cases of metallothionein binding of metals or metabolism and excretion of the pollutants. The effects may be histopathological, biochemical, physiological, behavioral or combinations of these. It is necessary for pollution assessment and abatement programs to identify which toxic effects on biota can be of immediate value as indicators of toxic contamination in estuaries. There is an especially pressing need for indicators that will give early warning of long term problems. The choice of effects to use at present must center around those that already have well developed methodologies. The effects should be easily observed and measured at affordable cost in order to serve as a screening system for pollution in the field. There are often a number of methodologies available for measuring the same or similar effects. Each of these should be considered and the most suitable for immediate use in pollution monitoring and abatement programs be given highest priority for refinement and field implementation at this time. The most promising methods for future use should be recommended to research and development arms of management agencies for continued support.

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