BIOINDICATORS

AS A MEASURE OF SUCCESS

FOR VIRTUAL ELIMINATION OF

PERSISTENT
TOXIC SUBSTANCES

BLACK-CROWNED NIGHT HERONS SNAPPING TURTLES LAKE TROUT WHITE SUCKERS BROWN BULLHEADS PHYTOPLANKTON BENTHIC INVERTEBRATES MIDGE MAYFLIES HUMANS MINK BELUGA WHALES BALD EAGLES HERRING GULLS DOUBLE-CRESTED CORMORANTS CASPIAN TERNS

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Report to the Virtual Elimination Task Force

BIOINDICATORS

AS A MEASURE OF SUCCESS FOR VIRTUAL ELIMINATION OF PERSISTENT TOXIC SUBSTANCES

A Report Based on a Workshop
Held April 28-29, 1992
at The Michigan League
Ann Arbor, Michigan

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

In 1978, the United States and Canada signed a new Great Lakes Water Quality Agreement for which the stated purpose was to "restore and maintain the chemical, physical and biological integrity of the waters of the Great Lakes Basin Ecosystem." The Agreement contains a policy on the discharge of toxic substances that "the discharge of toxic substances in toxic amounts be prohibited." This policy conforms with the well established regulatory approaches in the respective countries relative to the control of water pollution. The policy on persistent toxic substances states the "the discharge of any or all persistent toxic substances be virtually eliminated." This policy, when it was enunciated, was a significant departure from existing regulatory regimes, and called for extraordinary action apparently outside the existing regulatory framework.

In 1990, the International Joint Commission set up a Virtual Elimination Task Force to advise the Commission on a strategy for the implementation of the virtual elimination policy. The Commission requested the Task Force to work on appropriate indicators to track progress toward the virtual elimination policy and to demonstrate ecosystem restoration and protection.

The Great Lakes Water Quality Agreement contains provisions for the development of ecosystem objectives, and the Parties have set up an Ecosystem Objectives Work Group which has drafted general ecosystem objectives for Lake Ontario. The purpose of this report is limited and is to provide advice on specific indicators relevant to the policy on virtual elimination, rather than on general indicators of ecosystem "health" or "integrity" as is being undertaken by the Parties.

The Virtual Elimination Task Force held a workshop on indicators in Ann Arbor, Michigan, on April 28-29, 1992. The workshop participants noted the difference between the many measurements that are routinely made to determine concentrations of substances in sources and environmental samples, and the special category of measurements that can justifiably be called "indicators." The participants provided definitions to differentiate between indicator species, biochemical markers and biological effects, and criteria for the selection of indicators.

The report is premised on the basis that there has been extensive injury to fish and wildlife resources and to human health as a result of discharges and releases of persistent toxic substances to the Great Lakes. The purpose of the policy is to stop the injury to the resources and human health. This injury is intrinsically biological and therefore the indicators must be biological and should relate to what has been injured and to the cessation of the injury.

Significant improvements have recently been made in applying scientific principles to integrate information from a variety of sources to provide reliable statements about the causal relation between the injury to fish, wildlife and human health and exposures to specific persistent toxic substances. Acceptance of these causal statements by scientists and regulatory officials is a prerequisite for implementation of the extraordinary regulatory action required to deliver the policy on virtual elimination of persistent toxic substances and thereby to stop the injury.

The challenge at this time is to select indicators with a high degree of specificity for cessation of the injury caused by persistent toxic substances as a small class of compounds and as individual compounds within that small class. In addition, the indicators should be relatively uninfluenced by other factors such as physical and chemical habitat alterations, introductions of exotic species and pathogens, and overharvesting. At this point in time, the only potential indicators that fulfil these criteria are top predators such as the lake trout, snapping turtles, mink and otter, osprey and bald eagle, and certain fish-eating birds such as gulls, terns and herons. Where information is available, it shows that populations of these species have generally increased with declining exposures to persistent toxic substances and reproductive success has improved to levels closer to those in less contaminated inland sites.

A further challenge is to gain acceptance of these new biological indicators within the well established bureaucracies responsible for water quality. The traditional approach to water quality involves the preparation of water quality objectives for specific substances based on experimental determination of the most sensitive endpoint in the most sensitive species, and the analytically determined concentration of the substance in environmental samples or in bioassays. The premise is that if the concentration of the compound is less than the water quality objective the resource is protected from potential harm by the substance. The major drawback to this approach is that if actual injury is occurring, caused by a substance(s) that is undetected and, thus, for which there is no established water quality objective, the situation can remain unidentified for decades. This is what happened in the Great Lakes basin with respect to the injury to fish and wildlife resources and human health, caused by persistent toxic substances during the past 60 years.

There is thus a series of advantages to the use of indicator species to monitor effects as well as exposures. The monitoring of indicator organisms provides further verification of the relationship between the injury and the putative causal agent. Rather than relying on extrapolation from experimentally determined safe levels derived from surrogate species, it provides direct evidence that the resources are no longer being injured. Further, indicator organisms can integrate exposures to this small class of persistent toxic substances from multiple sources over a geographic scale relevant to the size of the Great Lakes and thus provide verification that the sources of the persistent toxic substance(s) have successfully been virtually eliminated.

In choosing these top predators as potential indicators of virtual elimination of persistent toxic substances there is a series of further questions to be addressed. Measurements to monitor these indicators are made at different levels of biological organization, ranging from the biochemical and ultrastructural levels through cellular, tissue and organ levels, to organisms and their populations within communities. As a general rule, effects are detectable at lower levels of biological organization with lower levels of exposure to the specific substances. This would suggest that all monitoring should be undertaken at the lowest level of biological organization practicable to ensure that effects are detected before there are collapses of populations. In the Great Lakes, the undetected damage was done several decades ago, and thus the priority is on monitoring at the organism and population level to document restoration of the extirpated species. As the concentrations of persistent toxic substances decline, and signs of effects are no longer overt, more subtle measurements are needed to minimize the amount of biological extrapolation between levels of biological organization, and the length of time to detect effects if there are further releases of persistent toxic substances.

Though certain populations of humans have been highly exposed to persistent toxic substances from the Great Lakes, it has proved difficult to undertake epidemiological studies, and thus to propose humans as indicators of virtual elimination of persistent toxic substances. Equally it has proved difficult to gain acceptance that the status of fish and wildlife populations can be used as surrogates of human health. The commonality of structure and function at the genetic, biochemical, cellular, tissue and organism levels of biological organization among vertebrates would suggest that there is no scientific basis for this difficulty.

Michael Gilbertson

1. INTRODUCTION

BACKGROUND

Considerable evidence has accumulated over the past two decades linking persistent toxic substances to injury, disease and death in a variety of life forms, including humans, in the Great Lakes basin. The most consistently observed effects in aquatic biota, particularly fish and fish-eating wildlife, are reproductive failure, population declines, developmental abnormalities, and generational effects. Grossly observable effects in these species include tumors, adult and embryonic mortality, deformities and other effects in offspring including functional deficiencies. Also observed are a number of biochemical and physiological changes whose biological significance is not yet fully understood (Government of Canada 1991). These changes may be subtle and may involve a breakdown in the homeostatic processes that sustain health and natural immunity, potentially altering the organism's ability to tolerate environmental change or to cope with disease.

The force driving the cleanup of the Great Lakes is the public desire to eliminate all these manifestations of toxicity. In response to this public pressure, the 1978 revision of the Great Lakes Water Quality Agreement calls for the virtual elimination of the input of persistent toxic substances into the Great Lakes. To contribute to the definition and resolution of the issue, the International Joint Commission established a Virtual Elimination Task Force to "provide advice and recommendations to the Commission about what a virtual elimination strategy should contain and how the strategy could be implemented". The Task Force "articulated a simple vision regarding persistent toxic substances: ecosystem integrity, characterized by a clean and healthy Great Lakes Basin Ecosystem and by the absence of injury to living organisms and to society" (Virtual Elimination Task Force 1993a). *The ultimate goal is to obtain and maintain a Great Lakes environment within which aquatic organisms, and those that feed on those organisms, including humans, are no longer affected by persistent toxic substances.*

In its Interim Report, presented at the Commission's 1991 Biennial Meeting, the Task Force recommended that "the Parties, with public consultation, select a suite of indicators and initiate measurement programs to track progress toward the Agreement goal to virtually eliminate the input of persistent toxic substances to the Great Lakes Basin Ecosystem" (VETF 1991).

The Commission focused on biological injury as justification for the need for virtual elimination of persistent toxic substances, and the Task Force focused on the absence of injury as a measure of the success of virtual elimination efforts. This report is a synthesis of a workshop held in Ann Arbor, Michigan on April 28-29, 1992, in which 33 experts representing a wide range of disciplines, backgrounds and perspectives attempted to identify a suite of indicators of biological injury that would be useful in tracking progress toward virtual elimination of persistent toxic substances. The assemblage did not include people with extensive experience with invertebrates and lower taxa; thus, the discussions focused on vertebrates and related manifestations of toxicity and biological injury.

Article I of the Agreement defines toxic substance as one "which can cause death, disease, behavioural abnormalities, cancer, genetic mutations, physiological or reproductive malfunctions or physical deformities in any organism or its offspring, or which can become poisonous after concentration in the food chain or in combination with other substances". In Annex 12, a persistent toxic substance is defined as "any toxic substance with a half-life in water of greater than eight weeks". Of the numerous persistent toxic substances known to be present in the Great Lakes Basin Ecosystem, the Commission's Great Lakes Water Quality Board has identified 11 Critical Pollutants, all associated with detrimental effects on biota and/or human health, as targets for virtual elimination (Table 1). The workshop focused on these substances.

TABLE 1. ***Critical Pollutants Identified by the Great Lakes Water Quality Board***

- Total polychlorinated biphenyl (PCB)
- DDT and its metabolites
- Dieldrin
- Hexachlorobenzene
- Toxaphene
- 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
- 2,3,7,8-tetrachlorodibenzofuran (TCDF)
- Mirex
- Benzo(a)pyrene
- Mercury
- Alkylated lead

THEORY

Chemical monitoring measures environmental contamination, often in relation to fixed criteria or guidelines. Although it may identify the *potential* for contaminant-related problems, it does not identify, quantify or describe these problems. Even if criteria or guidelines are met, there is no assurance that biota are protected from unknown effects, the effects of unidentified chemicals, or unexpected effects of geochemical processes, chemical mixtures, or interactions with other environmental stressors. On the other hand, biological monitoring describes the structure and function of communities, populations and individual organisms. Biota integrate spatial and temporal variations in exposure to the complex of stressors in their environment, and measures of their health are rarely diagnostic of a particular cause. Hodson (1990) concludes "that biological and chemical monitoring must be combined systematically using the principles of epidemiology to establish strong cause-effect relationships" and "the most efficient monitoring programs will integrate the measurement of chemical levels in biota and other environmental samples with measurements of the health of populations and signs of chemical effects in individual organisms".

The Interim Report of the Task Force identified the measurement of ambient concentrations of persistent toxic substances in sediments, benthic and other biota as an essential component of its virtual elimination strategy. The workshop did not address this aspect further.

THE NEED FOR INDICATORS

The ultimate goal of virtual elimination is to obtain and maintain a Great Lakes environment within which aquatic organisms, and those that feed on those organisms, including humans, are no longer affected by persistent toxic substances. Milestones leading to the achievement of this goal must be identified, assessed, and incorporated into the elements of the virtual elimination strategy. A suite of indicators which measure loadings, presence and toxicity of persistent toxic substances must be clearly identified.

Indicators are needed in a virtual elimination context to:

1. Establish the current status and trends of the Great Lakes Basin Ecosystem in regard to loadings of persistent toxic substances.

2. Track progress, over time, toward virtual elimination of persistent toxic substances within the Great Lakes Basin Ecosystem.

3. Demonstrate that virtual elimination of loadings of persistent toxic substances has been achieved and that health of biota and ecosystem function is not impaired by persistent toxic substances.

4. Ensure long-term protection of the ecosystem from persistent toxic substances after successful restoration.

BIOINDICATORS

Toxicity is an integrated biological response to exposure to a host of chemicals in an organism's environment. Bioindicators gauge toxicity. *A bioindicator is an organism and/or biological process whose change in numbers, structure or function points to changes in the integrity or quality of the environment.* In this report, bioindicators include:

- Biochemical markers, which use biochemical reactions to measure changes in cellular or subcellular processes.

- Biological effects, which are measurable changes in the development, behaviour, or success of the species.

- Indicator species, i.e. organisms whose biological characteristics enable meaningful quantitative measures of changes in structure or function to be made relatively easily.

These measurements become bioindicators when they help establish or demonstrate a cause-effect association between a persistent toxic substance and injury in a biological species. Chemical measurements tell us about the presence of contaminants in water, sediment, and fish. However, toxicity cannot be assessed merely by identifying and quantifying chemicals in various environmental media. Unlike chemical measurements, biological monitoring tells us whether contaminants are biologically available. Bioindicators provide a reliable measure of success in achieving the virtual elimination goal, rather than the attainment of some concentration established by calculation. The absence of gross and subtle manifestations of toxicity and the restoration of a functionally healthy ecosystem are the ultimate goals of the Great Lakes Water Quality Agreement.

It is through the use of bioindicators that research and resource managers will know whether the regulatory actions undertaken to control persistent toxic substances have been successful and whether the virtual elimination policy in the Agreement has been delivered.

WORKSHOP SCOPE AND OBJECTIVES

The objective of the workshop was to identify a series of bioindicators that, together, are necessary and sufficient to meet the four needs stated above. Since the goal is to ensure that the biological community, including humans, is not affected by persistent toxic substances, the indicators chosen must be biologically, rather than chemically, based.

Considerable work is presently underway in the United States, Canada, and elsewhere to identify suitable bioindicators. The Commission itself has sponsored and published the proceedings of a number of relevant workshops (CGLRM 1991, Best *et al.* 1990, Addison *et al.* 1991, Mac and Gilbertson, 1990). It was the desire of the Virtual Elimination Task Force to build upon what has already been considered and developed. Therefore, the workshop drew upon those initiatives which identify extant indicators that could be applied to the Great Lakes virtual elimination issue.

WORKSHOP ORGANIZATION AND PARTICIPANTS

On behalf of the Virtual Elimination Task Force, 33 experts, representing a wide range of disciplines, backgrounds, and perspective on the issue, met in Ann Arbor, Michigan on April 28-29, 1992. The workshop was organized to provide, firstly, perspective on the issue, through a series of five presentations:

· Background	Dr. John E. Gannon
· Biomarkers	Mr. Glen Fox
· Cause-Effect Linkages	Dr. James P. Ludwig
· Ecosystem Indicators	Dr. Dora R. Passino-Reader
· Ecological Equivalence Species	Dr. G. Douglas Haffner

The presentations were followed by three breakout sessions, which allowed extensive interaction and detailed discussion of these topics. The participants periodically reconvened in plenary session to review progress and, ultimately, to provide advice about the desired product.

Participants were asked to nominate bioindicators, review their pros and cons, and identify those which are necessary, most useful, and sufficient in the Great Lakes to support the virtual elimination goal of eliminating all manifestations of toxicity in fish and fish-eating wildlife. First, participants were asked to identify bioindicators which are immediately available for use and which are as specific as possible to measure the injury caused by persistent toxic substances. Participants were then asked to identify potential bioindicators for which research and development are required, in order to ensure their routine application in the context of biomonitoring/virtual elimination. In order to help identify the desirable characteristics of the species selected and the measurements to be made, the Workshop Steering Committee then developed a set of criteria against which to evaluate the potential indicators and species. The criteria are listed in Chapter 2.

WORKSHOP PRODUCT

The workshop product, presented in this report, consists of:

- A suite of bioindicators -- biochemical markers, biological effects and indicator species -- to be used in the context of virtual elimination of persistent toxic substances.
- The characteristics, criteria, and rationale for their identification and selection.
- Data needs, which will provide advice for surveillance and monitoring programs.
- Identification of initiatives through which indicators can be applied and the requisite information obtained.
- Advice on the collection, interpretation, and management of data. The advice is intended to ensure that the requisite information is available to track progress toward, and achievement of virtual elimination of inputs of persistent toxic substances and the cessation of toxicity.
- Research needs.

Prior to completion of this report, a draft, based on the deliberations at the workshop, was prepared by the Workshop Steering Committee and circulated to the workshop participants, the Virtual Elimination Task Force, the Commission's Council of Great Lakes Research Managers, Science Advisory Board, and Water Quality Board for review and comment. This present document takes into account the thoughtful and constructive insight provided by 20 respondees; these people are identified at the end of this report. The Workshop Steering Committee is grateful for the input provided by the workshop participants and the reviewers. However, the contents, conclusions, and recommendations contained in this report are those of the Steering Committee and not necessarily those of the participants, reviewers, Virtual Elimination Task Force, or the Commission.

THE AUDIENCE

The advice in this report is provided to the Task Force, but is intended to be useful for several target audiences. Firstly, the public are the beneficiaries of a healthy Great Lakes Basin Ecosystem. How can routine reports about bioindicators, which are not necessarily either glamorous or easily understood, be related to general goals such as fishable, swimmable, and drinkable waters, which the general public do understand and will support?

Secondly, regulators and environmental resource managers must be able to measure whether the injury is getting better or worse. Have their actions stopped the sources and improved, restored, and protected the ecosystem?

Thirdly, the scientific community requires bioindicators to measure the incidence and severity of the injury. Are we generating the right data? Do we have the right species, biochemical markers, and biological effects? What additional tests and procedures must be developed?

Lastly, policy makers, who ultimately decide on the goals for the Great Lakes, and on the allocation of resources to meet those goals, must be cognizant of the injury and the means to measure its amelioration.

2. SELECTION CRITERIA

The Workshop Steering Committee established four criteria -- or desirable characteristics -- for the selection of indicator species, biochemical markers and biological effects for measuring the success of a virtual elimination strategy for persistent toxic substances in the Great Lakes. These criteria, developed prior to the workshop, served as guidelines for the participants in their deliberations. These criteria are presented and discussed below.

SPECIFICITY (SENSITIVITY) TO PERSISTENT TOXIC SUBSTANCES

In its Interim Report, "Persistent Toxic Substances: Virtually Eliminating Inputs to the Great Lakes", one of the recommendations of the Virtual Elimination Task Force was "that the Parties immediately initiate measures to sunset the 11 Critical Pollutants, including all aspects of their manufacture, use, and disposal." The Task Force reiterated and amplified this advice in its Final Report (Virtual Elimination Task Force 1993a). These 11 Critical Pollutants (listed in Table 1), originally identified by the Water Quality Board in 1985, were targeted by the Task Force for virtual elimination from the Great Lakes, in compliance with the policy stated in the amended Great Lakes Water Quality Agreement.

The workshop was aimed at identifying and selecting bioindicators that measure effects associated with these critical pollutants in different species in the Great Lakes. ***In particular, emphasis was placed on those manifestations that have been previously reported in Great Lakes fish and fish-eating wildlife (Government of Canada 1991) which were the initial stimuli for virtual elimination.*** To facilitate deliberations, benzo(a)pyrene was expanded to include all polynuclear aromatic hydrocarbons (PAHs), and emphasis was placed on measurement of injuries attributable to DDE, PCBs+TCDD+TCDF, mercury, alkylated lead, and PAHs. More generally, the intent was to identify biological injuries that could be attributed to persistent toxic substances and, if possible, a particular persistent toxic substance or group of substances. *Ideally*, the measurements should be unequivocable, i.e. not confounded by factors such as land use and resource management practices, habitat loss, species competition or other biochemical explanations.

The 11 persistent toxic substances designated by the Commission and targeted by the Virtual Elimination Task Force are a small percentage of those known to be present in Great Lakes food webs. They are, however, those which have been associated with one or more toxic manifestations in biota or are known to be toxic from laboratory studies. Free-living fish and wildlife in the Great Lakes basin are exposed to most of these compounds as a complex mixture, in which certain compounds contribute more to the toxicity than others, but in which the interactive effects cannot be predicted or tested. Similarly, there are a limited number of biological expressions of toxicity, and the toxicity of many compounds may be manifest in the same way. To our knowledge, there are no biomarkers that have been studied in fish or wildlife that are specific for hexachlorobenzene, dieldrin, mirex, toxaphene, or alkyl lead. Mercury is a nephrotoxin and a neurotoxin, but neither form of toxicity has been specifically documented in Great Lakes biota. *Therefore, "specificity" in the context of this workshop implied a toxic chemically (rather than a biologically or nonchemically) induced response.*

PLACEMENT IN APPROPRIATE SCALES

The bioindicators to be selected for use in a virtual elimination context have to be characterized as to scale:

- The **organizational** (biological) scale, ranging from molecular through cellular, organism, population, assemblage to whole ecosystem.
- Their geographic distribution and abundance in different areas within the Great Lakes (**spatial**). For the Great Lakes, the geographic scale covers the Areas of Concern, whole lakes, and basin ecosystem.
- Their **temporal** characteristics for short-term or long-term monitoring.
- The position of the organism in the food web (**trophic**).

EASE AND COST OF MEASUREMENT

The bioindicators selected should be sensitive and simple to measure at a nominal cost and in a reasonable time frame. The question of randomized *vs.* selected sampling design should also be considered. The key factors are:

- **Measurability.** An available standard protocol, with low measurement error, is required to produce interpretable, valid results.

- **Cost-Effectiveness.** Once established, routine monitoring of the indicator should be accomplished with low maintenance and cost.

SOCIAL RELEVANCE/PUBLIC PERCEPTION

A socially relevant indicator would be of obvious value to and observable by shareholders or predictive of a measure that is. In a political context (CCRLM 1991), the success of any indicator species is dependent on whether the general public values it. In this regard, the bald eagle has become a powerful symbol of ecosystem integrity in the Great Lakes as well as being the national symbol of the United States of America. Indeed, the American anthropologist Margaret Mead suggested that the bald eagle can become an object of protective care.

3. CAUSE-EFFECT ASSOCIATIONS

Toxicity is an integrated biological response to exposure to the host of chemicals in an organism's environment and cannot be assessed by merely identifying and quantifying chemicals in environmental media. According to Reynoldson *et al.* (1989), "there has been a developing awareness that chemical objectives alone are insufficient as indicators of overall 'health' of the ecosystem, and that ultimately the biological integrity of the ecosystem is the prime concern". The ultimate measure of our success in achieving the Agreement goal of virtual elimination of persistent toxic substances will be the absence of gross and subtle manifestations of toxicity and the restoration of a functionally healthy ecosystem.

In his insightful paper on indicators of ecosystem health, Hodson (1990) concluded:

1. Traditional water quality monitoring is an inadequate way to monitor the health of aquatic ecosystems. Even when all water quality objectives have been met, biogeochemical processes and biomagnification can enhance accumulation and toxicity of chemicals.

2. Population monitoring provides an important, highly relevant first indication of impaired environmental quality, but is a poor basis for diagnosing cause. The lag between identifying a problem and finding a cause may destroy the resource we wish to protect, particularly where chemicals are persistent. [*While population level responses in fish are not diagnostic, such responses in phytoplankton and benthic invertebrates are very rapid and can be quite diagnostic, even at the community level. Plankton and benthos may have similar response times at the population and community level as biochemical changes in fish and wildlife.*]

3. The biochemical, physiological and pathological responses of individual organisms, when coupled with chemical measurements, provide an excellent basis for diagnosing chemical exposure and effects.

4. The significance of highly specific biochemical and pathological responses at the suborganismal level to populations and ecosystems is poorly understood. There is a need to study links between chemical exposure and responses of individuals, populations and ecosystems.

5. The most efficient monitoring programs will integrate the measurement of chemical levels in biota and other environmental samples with measurements of the health of populations and signs of chemical effects in individuals. The strength of proposed cause-effect relationships can be established by applying epidemiological criteria. Where a poor "fit" is observed or where a question can not be answered, there is a clear definition of a research need.

If we are to use bioindicators to monitor our progress towards virtual elimination of persistent toxic substances, and the justification for virtual elimination is based on the manifestations of toxicity observed in Great Lakes fish and fish-eating wildlife, then we must monitor for those manifestations, particularly those for which a causal association has been established with the persistent toxic substances of concern (Table 2), and be prepared to test other cause-effect associations we believe exist.

According to Lilienfeld and Lilienfeld (1980), ***"a causal relationship would be recognized to exist wherever evidence indicates that the factors form part of the complex of circumstances that increases the probability of the occurrence of the disease and that a diminution of one or more of these factors decreases the frequency of that disease".***

Observations of the apparent effects of contaminants on free-living fish and wildlife are always correlational. The potential causal agents considered are those we measure or observe, a subset of those present -- imperfect knowledge at best. Free-living organisms are exposed to a number of contaminants and stressors and the biomarker values we measure are the system's integrated biological response to that suite of stressors. Sutter (1991) warns us not to fall prey to what is known to epidemiologists as the "ecological fallacy" -- the idea that occurrence of an effect in conjunction with a plausible environmental factor proves that factor is the cause. Before a cause-effect association can be established, a systematic evaluation of the evidence, using the following epidemiological criteria (Susser 1986, Fox 1989) is required:

- **Time-Order**. Does cause precede effect in time? This may be difficult to establish in systems with little historic data.

TABLE 2. ***Some Contaminant-Associated Injuries and Impairments Documented in Fish and Wildlife in the Great Lakes Basin***

POPULATION	
Declines	Fish, fish-eating birds, beluga, (mink)
Recruitment alterations	Fish, fish-eating birds, beluga
Skewed sex ratios	Fish-eating birds
INDIVIDUAL	
Mortality	Fish-eating birds, beluga
Behavioural abnormalities	Fish-eating birds
Growth	Fish, fish-eating birds
Reduced reproductive success	Fish-eating birds, beluga
Developmental toxicity	Fish, snapping turtle, fish-eating birds
CELLULAR/SUBCELLULAR	
Induction of detoxication system	Fish, fish-eating birds
Inhibition of specific enzymes	Fish, fish-eating birds
Metabolic impairments	Fish-eating birds
Genetic damage or impaired repair	Fish-eating birds, beluga
Impaired immune function	Fish-eating birds, beluga
Endocrine disruption	Fish, fish-eating birds, beluga
Neoplasia	Fish, beluga

- **Strength of the Association**. Do cause and effect coincide in their distribution? Is the effect large relative to the cause? Is the prevalence of the effect in exposed populations large relative to unexposed populations?

- **Consistency of the Association**. Has the association been repeatedly observed in different places, circumstances, times, and species, or by other investigators with different research designs?

- **Coherence of the Association**. Is the cause-effect interpretation consistent with our current understanding of the biological mechanism(s) underlying the effect? Is an exposure-response relationship present? Do laboratory studies support the proposed relationship? Do remedial actions lead to altered frequency or severity of the effects?

- **Specificity of the Association**. Could the effect be due to a different cause? Could the proposed cause produce other effects? Can alternative hypotheses be eliminated? In the context of the Great Lakes, where a multiplicity of persistent toxic substances and ecological perturbations are present, specificity may be complicated by chemical interactions, commonality of mode of action, and interspecific differences.

- **Predictive Power of the Association**. A hypothesis drawn from an observed association predicts a previously unknown fact or consequence, and must in turn be shown to lead to that consequence.

What is the nature of the evidence that must be ignored to conclude that no causal relationship exists? What alternative explanation will fit our observations and what other differences between our contrasted groups could equally, or better account for the observed incidences? Failure to satisfy all of the epidemiological criteria does not necessarily negate the hypothesis, but may instead point to significant gaps in knowledge that may be resolved in the course of future studies (i.e. we are bound by the imperfect knowledge of our time). Lack of fit may be a definition of a research need.

Cause-effect associations which are epidemiologically consistent should be experimentally confirmed, using extensions of Koch's postulates for proving that a particular pathogen causes a disease, i.e.

- Controlled exposure of susceptible organisms to a concentration gradient of the chemical, complex effluent, or contaminated medium that is associated with the effect in the field, results in the effect in an exposure-related fashion.

- Measures of exposure (i.e. body burden) in field studies and in the controlled toxicity tests establish that the organisms in the field are exposed to the pollutant and that the degree of exposure is consistent with the degree of exposure that causes the effect in the laboratory.

4. BIOINDICATORS: BIOCHEMICAL AND BIOLOGICAL MEASURES OF EXPOSURE AND EFFECT

BIOCHEMICAL MARKERS

Introduction

Health of an organism can be defined as the residual capacity to withstand stress; the more stressed, the less capable is the organism of withstanding further stress (Bayne *et al.* 1985). Brett (1958) defined stress at the individual level of ecological organization as "a state produced by an environmental or other factor which extends the adaptive responses of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced". A stressor is any condition or situation that causes a system to mobilize its resources and increase its energy expenditure (Lugo 1978). Depending on its severity, sublethal stress may limit physiological systems, reduce growth, impair reproduction, predispose the individual to infectious diseases, and reduce the capacity of the organism to tolerate additional, normal stressors.

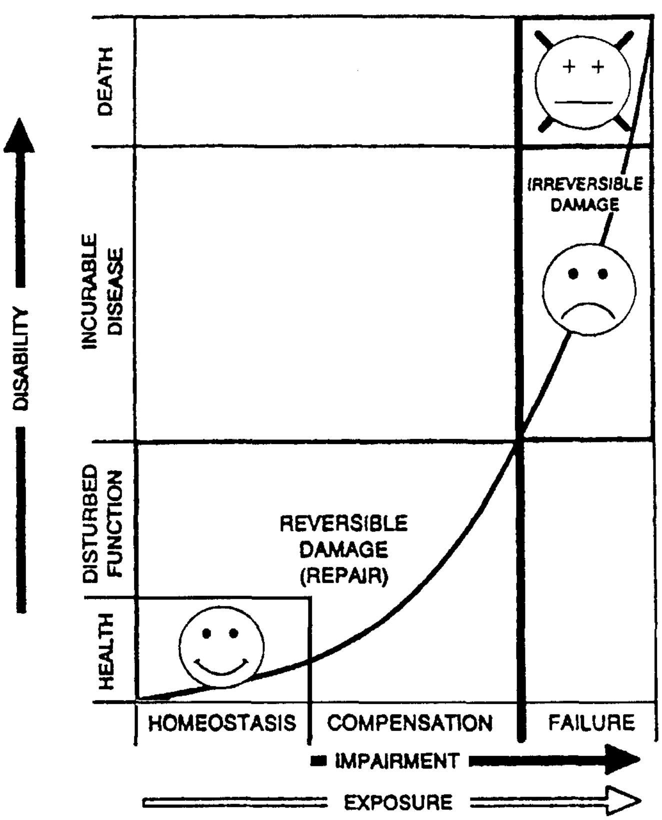
It was Hatch (1962), an occupational health specialist, who first distinguished between "impairment" -- the underlying disturbance of the system or precursors of disease, and "disability" -- the consequences of such disturbance in terms of identifiable disease. Depledge (1989) recognized the fundamental importance of Hatch's work to physiological monitoring in ecotoxicology. It is apparent from studies of the effects of stress and disease in biological systems, that there is a gradient, starting with normal health or homeostasis, progressing along scales of impairment and disability, ultimately to failure or death (Figure 1). Both Hatch (1962) and Depledge (1989) concluded that measures of impairment are more sensitive to pollutant effects than are measures of disability. Hence, monitoring the underlying biochemical alterations and impairment of physiological and behavioural responses will clearly provide early warning of the onset of disabilities and provide an understanding of the specific mechanism(s) by which health was impaired. In this report, we call these sensitive sublethal indicators, the precursors of disease, biochemical markers.

Biochemical markers are indicators measured in a biological system which can be related to exposure to, or effects of, a xenobiotic compound. They are measures of the rates of chemical reactions or the amounts of biochemical products in cellular or subcellular systems, sublethal biological effects, or histological measures that relate in a dose- or time-dependent manner, the degree of dysfunction that the contaminant(s) has produced. Biochemical and biological markers of effect include:

- Induction of the stress response.
- Induction of detoxication systems.
- Inhibition of specific enzymes.
- Metabolic impairments that detrimentally alter synthetic or degradative processes or that deplete energy, vitamin, or substrate stores.
- Genetic damage or altered repair.
- Impairment of immune system function.
- Impaired or altered reproductive function.
- Impaired organ or tissue function based on functional tests or histopathological alterations.

Because biochemical changes can be used to assess exposure to toxic chemicals and the associated biological effects, biochemical markers are gaining popularity for application in the Great Lakes ecosystem. These measurements are a sensitive and essential component of modern environmental assessments. They indicate

FIGURE 1 ***The Exposure-Response Curve***



The relationship between "impairment" and disability" in pathophysiological responses to increasing contaminant exposure in biological systems.

where a measurable, dose-related physiological or biochemical change takes place at the molecular or cellular level upon exposure to a toxic chemical, and thus provide vital information on mechanisms of toxic action. The target molecules for many toxic substances appear to be similar among organisms, including man. Hence, studies of biochemical changes in different hierarchic levels should be valuable in assessment of hazards to human health.

The biochemical methods developed in this category all have the basic premise that *"toxic effects begin with a reaction between the toxic chemical and some biochemical receptor in a living organism"* (NRC Canada 1985). If a good battery of biochemical tests is available covering the major functions of an organism, then it is possible to assess whether the parameters measured for an individual are within *normal limits* for the species or differ significantly from individuals of the same species from a "cleaner" physiologically and ecologically comparable site(s). For our purposes, a useful biochemical marker:

- Should be relatively easy to measure, allowing quantification of multiple individuals.
- Will have variability due to other factors (e.g. season, temperature, sex, weight, handling) that is understood and within acceptable limits.
- Will respond to persistent toxic substances of concern in a dose-dependent manner over a concentration range which is environmentally relevant.
- Should allow some extrapolation of harm to the individual (and population).

Although many of these biochemical markers differentiate between stress induced by persistent toxic substances and confounding factors such as habitat loss, interspecies competition, and food shortage, *there are, however, few biochemical markers that are specific for a particular persistent halogenated toxic substance. Rather, the biochemical markers chosen tend to be associated with groups of persistent toxic substances by their mode of action or the measurable biochemical outcome of such action.* Table 3 tabulates biochemical markers which can be associated with persistent toxic substances identified for virtual elimination.

Although biochemical measures are increasingly receiving attention and acceptance for their ability to indicate exposure to, and effects of, toxic chemicals, they lack public appeal unless they can be related to a biologically or sociologically relevant effect on survival, reproduction, or population size or condition. It is far more challenging to get the public excited about declining MFO enzyme activity in bald eagles than the return

TABLE 3. ***Biochemical Markers Affected by Persistent Toxic Substances of Concern***

BIOCHEMICAL MARKER	PERSISTENT HALOGENATED AROMATIC HYDROCARBONS	PAHs	TOXIC METALS
MFO Induction			
EROD, AHH, CBT (P450-1A1)	TCDD(F), some PCBs, hexachlorobenzene (HCB)	+	-
PROD, BROD (P450-2A)	DDT, some PCBs, dieldrin, toxaphene	-	-
Altered Vitamin A Storage	TCDD(F), PCBs, DDT, dieldrin	(+)	-
Altered Porphyrin Patterns	PCBs, TCDD(F), HCB, octachlorostyrene (OCS)	-	-
Altered Thyroid Function	PCBs, TCDD, DDT, HCB, OCS, mirex dieldrin	(+)	-
DNA Alterations	-	+	-
Liver Tumors	-	+	-
ALAD Inhibition	-	-	Pb
Neurotoxicity	DDT, dieldrin, some PCBs	-	Hg, Pb
Nephrotoxicity	-	-	Hg, Cd
Altered Bone Strength	Toxaphene, PCBs	-	-

Note: Acronyms are identified in the text.

and successful breeding of these eagles on the Great Lakes shorelines. New methods for presenting data of this nature to the public could be developed as biochemical research becomes more established. Relevance of these data to our daily lives is an essential component. Some of these tests are analogous to diagnostic human blood and urine tests, which compare the cholesterol, sugar and protein levels in human specimens as a warning signal for the onset of harmful diseases such as heart disorders, diabetes, and liver *sclerosis*.

In considering biochemical measurements for use in a virtual elimination context, the strategy at the workshop was to categorize candidate biochemical markers, considering the associated organizational, temporal, spatial, and trophic scales; their specificity (sensitivity) to persistent toxic substances; their applicability to particular indicator species; and the ease and cost of collecting and analyzing samples to obtain data. The discussions centered on available biochemical markers for use immediately and the indicator species in which they have or could be used. Immunotoxicology and neurotoxicology were identified as being in a "research mode" which means that development is pending soon. Biochemical markers for nephrotoxicity and osmoregulation were identified as "research needs". The biochemical markers which the group thought are ready for immediate use include Cytochrome P450s (for fish, birds, and mammals), Vitamin A (mammals, birds, and fish), porphyria (mammals, birds, and maybe some fish), thyroid function (fish, birds, and mammals), and DNA damage (fish, birds, and whales). These are discussed below.

Induction of Cytochrome P450

The induction of cytochrome P450 enzymes is a broad adaptive response involving altered activities of many enzymes (mixed function oxidases or MFOs). MFOs are major components of the biological defence of living organisms against intrinsic and extrinsic chemical environmental stresses. In general, these enzymes add oxygen to a wide variety of endogenous and xenobiotic compounds, including hydrophobic aromatic hydrocarbons, making them more water soluble and more readily excreted by the body. However, the metabolites of some xenobiotic compounds are more toxic than the parent compound and the outcome unfavourable. Exposure to significant quantities of chemicals such as PCBs can result in induction of enzymes which may increase the toxicity of other contaminants and alter the rate and patterns of normal biosynthesis and metabolism of essential biomolecules such as steroid hormones and prostaglandins. MFO enzymes are present in a wide range of organisms including humans and since they play a central role in detoxication, they can be good biomarkers of exposure to xenobiotic chemicals (Rattner *et. al.* 1989).

Various forms of cytochrome P450 can be induced by all nine of the persistent organic substances targeted for virtual elimination (see Table 1); however, some are more potent inducers than others. Coplanar PCBs, TCDD and TCDF, HCB and PAHs all induce cytochrome P450 isozyme 1A1 (measured by AHH or EROD activity) while BROD and PROD activities reflect induction of other isozymes, induced by DDE, some PCBs, dieldrin, and toxaphene. AHH, EROD, BROD, and PROD assays are well established and AHH and EROD have sufficient field verification. MFO assays have been applied to species ranging from planaria to humans; mammal, bird, and fish data are extensive. MFO activities are typically determined on microsomal fractions of hepatic or other tissue homogenates obtained by ultracentrifugation or gel filtration. The assays are relatively easy and inexpensive to run. The caffeine breath test (CBT) is a noninvasive method of measuring cytochrome P450-1A1 activity that has been widely used in toxicological investigations of exposed human populations and has been validated in the laboratory (chickens) and field (herring gulls) for birds (Lindley *et al.* 1993 and pers. comm.). Two of the most commonly used measures of MFO enzyme activity are discussed in more detail below.

Aryl Hydrocarbon Hydroxylase (AHH)

AHH is a monoxygenase isozyme of the cytochrome P450-1A1 system. Induction of AHH activity reflects binding of the contaminant to the cytosolic aryl hydrocarbon (Ah) receptor which is a necessary, but not sufficient, step (Poland and Knutson, 1982) in the mode of toxic action of TCDD, TCDF, coplanar PCBs, HCB, and PAHs. In laboratory studies, Ah receptor binding has been associated with weight loss, edema, hepatotoxicity, immunotoxicity, reproductive toxicity, promotion of tumors, and cytochrome P450-1A1 induction. Many of these toxicities are consistent with those observed in wildlife and fish in the Great Lakes basin. TCDD is the most potent growth dysregulator known, and in laboratory studies, cytochrome P450-1A1 induction, immunotoxicity and reproductive effects all can occur at similar low doses (Birnbaum, in press). There is therefore sound biological support for using Ah receptor activation, as indicated by cytochrome P450-1A1 induction measured as AHH or EROD activity, both as a biomarker of polyhalogenated aromatic hydrocarbon exposure and toxicity.

The AHH assay is based on the amount of fluorescence produced by AHH-induced hydroxylation of benzo-a-pyrene. In most Great Lakes biota, two PCB congeners (3,4,3',4'-tetrachlorobiphenyl and 3,4,5,3',4'-pentachlorobiphenyl) generally account for over 90% of the estimated median toxicity in Great Lakes samples, as measured by AHH induction and 2,3,7,8-TCDD represents less than 5% of the toxicity. However, it is possible that an unknown portion of this activity is a response to as yet unidentified components.

Ethoxyresorufin O-deethylase (EROD), Benzyloxyresorufin O-deethylase (BROD) and Pentoxyresorufin O-deethylase (PROD)

EROD, another cytochrome P450-1A1 isozyme, exhibits similar metabolic action to AHH. The assay is simpler, safer, and less expensive to perform than the AHH assay and is currently more widely used as a biomarker of exposure and effect in fish and wildlife studies. Recently it has been shown that certain unidentified, water soluble, nonchlorinated, nonpersistent molecules in some pulp mill effluents can induce EROD activity in fish. In some Lake Superior white suckers, this induction has been associated with other metabolic and reproductive abnormalities (Munkittrick *et al.* 1992; Carey *et al.* 1993).

The EROD, BROD and PROD assays differ in substrates but are performed in a similar fashion and have been adapted to microplates and semiautomation

Both EROD and AHH induction are frequently expressed in 2,3,7,8-TCDD equivalents, in which 2,3,7,8-TCDD is given a value of 1 since it is the most potent inducer known, on a weight basis, of all xenobiotic compounds. This allows a summation of the Ah receptor-mediated activity of the various inducers present. However, the relative potency of various congeners differs in fish, birds and mammals, and between species.

Vitamin A (Retinol) Storage

Liver and, to a lesser extent, serum and yolk concentrations of retinol and retinyl palmitate have been identified as another field-validated biochemical marker. Retinol is the major natural form of Vitamin A, which is essential for normal vison, reproduction, cellular immune function, and maintenance of differentiated epithelia and mucous secretion in higher vertebrates. Over 95% of the body's retinoid reserves are stored in the liver, predominantly as the fatty acid ester retinyl palmitate. This ester may be hydrolyzed to retinol, depending on dietary intake and physiological requirements.

According to Zile (1992) normal Vitamin A function depends on adequate stores of the vitamin, a finely regulated supply of the vitamin to the target tissues, and an ability of cells to generate functionally active forms of the vitamin. Dietary intake and other exogenous factors can alter Vitamin A homeostasis. It is well established that dietary exposure to xenobiotic chemicals including PCBs, PBBs, TCDD, DDT, dieldrin, and PAHs can "cause servere disturbances in Vitamin A metabolism, manifested by accelerated metabolism and breakdown of Vitamin A and its metabolites and a depletion of Vitamin A from the body; this sequence of events accounts for the Vitamin A deficiency-like sysmptoms associated with PHAH intoxication" (Zile 1992). Vitamin A storage is therefore a very sensitive marker for nutritional status and the toxicity of PCBs and other polyhalogenated aromatic hydrocarbons (PHAHs).

Brouwer and Van den Berg (1984) have suggested that reduction in retinoid stores is a very sensitive marker for the toxicity of PCBs and related compounds. It is probably the most sensitive of those biomarkers which have been field-validated. Field studies have shown that liver Vitamin A stores were inversely related to the level of 2,3,7,8-TCDD. Rat studies have shown that polyhalogenated biphenyl congeners accelerated the conjugation and hydroxylation of retinoic acid in the liver, and bound components in the Vitamin A blood transport system. As with induction of cytochrome P450, reduction in exposure is accompanied by an increase in stored Vitamin A.

Much work has been done on mammals and birds but work on fish has been identified as a research need. Recently, investigators at the Freshwater Institute (Canada Department of Fisheries and Oceans) have reported that a single oral dose of the coplanar PCB 126 reduced both plasma and liver retinoid levels in immature lake trout (Brown *et al.* 1993) and that liver retinol and retinyl palmitate were decreased in feral lake trout, but not white suckers, one year after a single oral dose of 1 ng/g 2,3,4,7,8-PCDF (Delorme *et al.* 1993). Tissue extracts are inexpensively analyzed using reverse-phase high-pressure liquid chromatography (HPLC), and critical standards are available from commercial vendors.

Hepatic Porphyrin Patterns

The porphyrias are a group of disorders in which inborn or chemically induced derangements of the enzymes involved in heme biosynthesis result in an alteration in the size and/or composition of the porphyrin pool. Hepatic porphyrin patterns are another biochemical marker which is already field validated. Determination of porphyrin patterns (i.e. relative amounts of uroporphyrin, hepta- and hexacarboxylic acid porphyrins, coproporphyrin, and protoporphyrin) and their concentrations, offers promise as a specific and sensitive biomarker for persistent halogenated aromatic hydrocarbon-induced toxicity. In birds and mammals porphyrin patterns are affected by PCBs, TCDD, TCDF, HCB, and octachlorostyrene (OCS). The majority of wildlife data have been generated for herring gulls; data go back as far as 1974 for this species (Fox *et al.* 1988, Kennedy and Fox, 1990). However, porphyrin patterns have been measured in other species of birds, and in mammals and fish. In the laboratory, the metabolic derangements disappear when sensitive organisms are no longer exposed and the herring gull data from a number of Great Lakes colonies show good temporal correspondence between PCB levels and levels of highly carboxylated porphyrins. Within-colony comparisons between 28-day old chicks and adult herring gulls suggest that this lesion is a marker of chronic exposure.

Sample preparation and analysis requires more "chemical finesse" than other biochemical techniques. Porphyrins are generally extracted with perchloric acid/methanol, concentrated on a disposable Sep-pak C_{18} cartridge, separated by HPLC and detected fluorometrically.

Thyroid Function

The thyroid is an initiator, integrator and modulator of various physiological processes, particularly those involving metabolism, development, differentiation and growth. This key role in essential processes makes thyroid function a highly relevant biochemical marker, but one which is also sensitive to a variety of environmental stressors.

Abnormal serum thyroxine concentrations, interference with receptor binding and transport of thyroxine, and altered ratios of bound versus free thyroid hormones are changes associated with xenobiotic compounds. These may lead to changes in metabolic and other physiological processes. These measurements, plus thyroid mass and histopathology all are measures of thyroid function. Thyroid function is affected by PCBs, TCDD, DDT, dieldrin, mirex, OCS, and chlorinated benzenes, and can be assessed in any vertebrate.

Thyroid hormone assays can be done inexpensively by most veterinary diagnostic laboratories. The radioimmunoassay (RIA) and enzyme immunoassay (EIA) procedures used are very well standardized and available commercially. However, thyroxine levels in birds are very low and assays have to be adapted by modifying the standard curves and sensitivity to cover this more restricted range.

DNA Alterations

PAHs are the persistent toxic substances present in the Great Lakes that are most frequently associated with damage to genetic material. Several tests are available to measure such damage. Probably the most frequently applied and relevant measures for the presence and effects of PAHs are the estimation of levels of fluorescent aromatic compounds (FACs) in bile and the prevalence of liver and skin tumors and lip papillomas in bottom-feeding fish species. There have been a number of studies using these measures in the Great Lakes.

The formation of a reactive metabolite-DNA adduct is often the significant event in the genetic toxicity of chemicals. DNA adducts, particularly those involving benzo-a-pyrene may be detected in tissues and have been found in the brains of beluga from the Saint Lawrence River. Some toxic chemicals cause breaks in DNA strands, either directly or indirectly, resulting in alterations of the molecule which may lead to transcription problems. The alkaline unwinding assay is a sensitive technique which has been used to detect and quantify DNA strand breaks in cell cultures by chemical carcinogens and physical agents, and has been applied (Shugart 1988) to fish, birds, and turtles. More research and field validation are required in the area of genetic toxicology of fish and wildlife.

Aminolevulinic Acid Dehydratase (ALAD) Inhibition

Delta-aminolevulinic acid dehydratase (ALAD) is a cytosolic enzyme active in the synthesis of hemoglobin. Exposure to lead causes a dose-dependent decrease in erythrocyte ALAD activity in free-living fish (Haux *et al.* 1986, Hodson *et al.* 1977), birds (Drash *et al.* 1987; Scheuhammer 1987, 1989) and mammals (Mouw *et al.* 1975). ALAD inhibition is a specific biomarker for lead exposure and has some value as a measure of toxicity. ALAD has been measured in herring gulls, white suckers and lake trout in the Great Lakes. Assays of ALAD are relatively simple, inexpensive, accurate and precise. Baseline ALAD activity is quite variable among unexposed individuals; therefore, the ALAD reactivation technique (ALAD activity ratio) is recommended since it allows each individual to serve as its own control.

BIOLOGICAL EFFECTS

Various published sources have examined the relationship between contamination by persistent toxic substances and the resulting biological and biochemical effects in Great Lakes fish and wildlife (Gilbertson 1989, Schneider and Campbell, 1991). Bioeffects include tumors; embryotoxicity and deformities; egg-shell thinning; growth retardation or failure to thrive; unexplained mass loss; functional biochemical disorders; and structural and functional changes in populations, communities, and ecosystems. These effects are manifested in fish, birds, and mammals. These biological effects often occur after a relatively long (chronic) exposure time, or at higher concentration levels of the contaminant.

It has been suggested by several authors that the results of population monitoring are the ultimate indicators of ecological effects. Schindler (1987) in his seminal paper on detecting ecosystem responses to anthropogenic stress concluded that "among population-level approaches, life-table population studies of invertebrates appear to be the most sensitive early indicators of stress in aquatic ecosystems". Munkittrick and Dixon (1989b) consider a population of fish found to be growing, reproducing, and surviving within the limits of a comparable reference population to be free from detrimental contaminant exposure effects. Temple and Weins (1989) have suggested that, in birds, the primary population parameters of birth rate, death rate, and rate of dispersal are most clearly tied to environmental changes. However, population responses frequently do not provide an indication of their cause, and McCarthy (1990) cautions that "since fecundity is a key parameter linking individual responses and ecosystem processes, particular attention should be directed at evaluating the molecular, biochemical, and endocrinological factors regulating reproductive capacity of an individual organism".

Growth

Alterations in growth are a nonspecific and relatively insensitive measure of stress. Determination of growth of prefledged fish-eating birds is possible, but unless birds are banded as nestlings, adults can not be aged. However, indices of body condition can be derived from body mass and various linear measures. Growth of individual fish can be quantified for prolonged periods because the scales and/or otoliths provide a marker of age. Growth rate and condition factor are both components of the Munkittrick and Dixon (1989b) scheme for assessing the health of aquatic ecosystems.

Demography and Reproductive Success

In birds, the subcomponents of the primary population parameters which can be measured are clutch size, hatching success, and fledging success. In addition, such measures as clutch volume, chick growth, and incidence of congenital abnormalities also provide information on environmental quality, food availability, and the presence of developmental toxins in the food chain. Such data are likely to be obtained in intensive, local species-specific studies involving individual marking of nestlings and trapping, color-banding and resiting breeding adults, allowing the survival and recruitment of cohorts to be calculated. Henny (1972) used a model incorporating mortality rate, recruitment rate, and age at sexual maturity to detect changes in population dynamics attributable to organochlorine pesticides in five of 16 species examined. All changes detected were in recruitment rate. Recent studies of bald eagles nesting on Great Lakes shorelines suggest that the turnover rate of breeding adults is excessive, reflecting increased adult mortality. The Caspian tern populations of the Great Lakes have been the subject of large-scale, long-term banding efforts which suggest differences in recruitment patterns between colonies in Canadian and United States waters (Ludwig 1979, L'Arrivee and Blokpoel, 1988) and a significant negative correlation between mean concentrations of PCBs in the plasma of breeding adults and the proportion of young recruiting to their natal region has been reported (Mora *et al.* 1993).

There are a limited number of ways in which stressors can affect fish population structure; population responses to contaminants should be identical to any non-specific, density-independent stressor. Population characteristics such as fecundity, mean age, and growth rate integrate the experience of the population over a relatively longer period of time into factors which can be easily measured (Munkittrick and Dixon, 1989b). Age class distributions of fish provide an indirect measure of recruitment and survival of various cohorts. Age and growth analysis, condition factor, and fecundity are standard methods in fisheries science. Growth, fecundity, and maturity are phenotypic expressions induced by the environment. Munkittrick and Dixon (1989a) use relative changes in mean age, condition factor, and fecundity in white suckers in their simple, inexpensive approach to assess ecosystem health. Similarly, Ryder and Edwards (1985) use harvest data, age at maturation, age distribution, growth rate, and other measures in lake trout in their "dichotomous key to ecosystem well-being".

Measures of reproductive success of populations are probably the most sensitive biological effects at the population level. Age at maturation, fecundity, and egg size are frequently used measures of reproductive effort in fish. Most of the persistent toxic substances of concern are known to alter reproductive success. In the field, reproductive success (survival of individual reproductive units to a particular state or degree of independence) is most easily measured in birds, but can be measured in artificially incubated eggs of fish, frogs, or turtles. Standardized methods must be developed and adopted for various species if this measure is to be used for monitoring purposes.

Developmental Toxicity

The manifestations of developmental toxicity are growth retardation, functional disorder, malformation, and embryo death. These outcomes often increase in frequency and severity as exposure to toxicants increases. Fox *et al.* (1991) suggest that monitoring sensitive sentinel populations of fish-eating birds (and other nonhuman members of the fish-eating guild) would assist in the detection of developmental toxicants in Great Lakes food chains. Most of the persistent toxic substances of concern are known developmental toxins. Manifestations of developmental toxicity have been observed in birds (embryonic growth retardation and death, crossed or abnormal bills, missing eyes, extra digits or otherwise abnormal feet or legs, hip dysplasia, feminization), fish (bilateral fin ray asymmetry), reptiles (embryonic growth retardation and death; deformities of the tail, hind- and forelimbs, carapace, cranium and upper and lower jaws; missing eyes and claws in snapping turtle embryos and hatchlings) and mammals in the Great Lakes basin and should be considered as very useful bioindicators at the individual organism level. The use of prevalence determinations for monitoring purposes will require the adoption of a standard protocol.

INDICATOR OR SENTINEL SPECIES

Description

The groundwork on indicator species was done by the Ecosystem Objectives Committee (formerly the Aquatic Ecosystem Objectives Committee) of the Great Lakes Science Advisory Board of the International Joint Commission. The terms of reference of the Board's Work Group on Indicators of Ecosystem Quality were to "appraise, evaluate and critique the feasibility of using an indicator/integrator organism as a surrogate of the state of health of the Great Lakes" (Ryder and Edwards, 1985). The terms of reference were ambiguous as to whether the indicators were for general "ecosystem health" or to be chosen with a high degree of specificity for persistent toxic substances pursuant to the Great Lakes Water Quality Agreement.

Ryder and Edwards classified species as either Type I or Type II indicator organisms.

> Type I are "specialized organisms that have narrow tolerances for most environmental properties. Type I indicator organisms are stenoecious, having evolved to be specially adapted for the pristine, but somewhat austere conditions originally found in naturally occurring systems such as the oligotrophic environment of the Upper Great Lakes.... Examples of such indicator organisms for oligotrophic systems may be lake trout (*Salvelinus namaycush*), deep-water sculpins (*Myoxocephalus quadricornis*), and the amphipod *Diporeia [Pontoporeia] hoyi*. Walleye (*Stizostedion vitreum vitreum)* and the burrowing mayfly (*Hexagenia limbata*) that thrive in mesotrophic systems are somewhat less stenoecious yet are sufficiently sensitive to certain cultural stresses or suites of stresses." Other examples of Type I species are bald eagle (*Haliaeetus leucocephalus*), Forster's tern (*Sterna forsteri*), Caspian tern (*Sterna caspia*), otter (*Lutra canadensis*), and beluga whale (*Delphinapterus leucas*).
>
> Type II are "less specialized organisms that have relatively broad tolerances for many environmental properties. Type II indicator organisms constitute a group known as euryecious organisms.... Some examples of euryecious organisms include carp (*Cyprinus carpio*), sludgeworms (*Limnodrilus* sp.), bloodworms (*Chironomus* sp.), *Cladophora* (a green alga), and *Microcystis* (a blue-green alga)." Other examples include mink (*Mustela vison*), herring gulls (*Larus argentatus*), white sucker (*Catostomus commersoni*) and brown bullhead (*Ictalurus nebulosus*).

The organisms that will be the most useful indicators of virtual elimination of persistent toxic substances are Type I organisms. Ryder and Edwards (1985) provide a detailed description of the requirements and definition of Type I organisms. As summarized by Edwards and Ryder (1990) "in order to qualify as a suitable [Type I] surrogate species, an organism must satisfy a minimal set of criteria: be a strong integrator of the biological food web at one or more trophic levels; be abundant and widely distributed within the system; and be one of perceived human value such that it may be easily sampled."

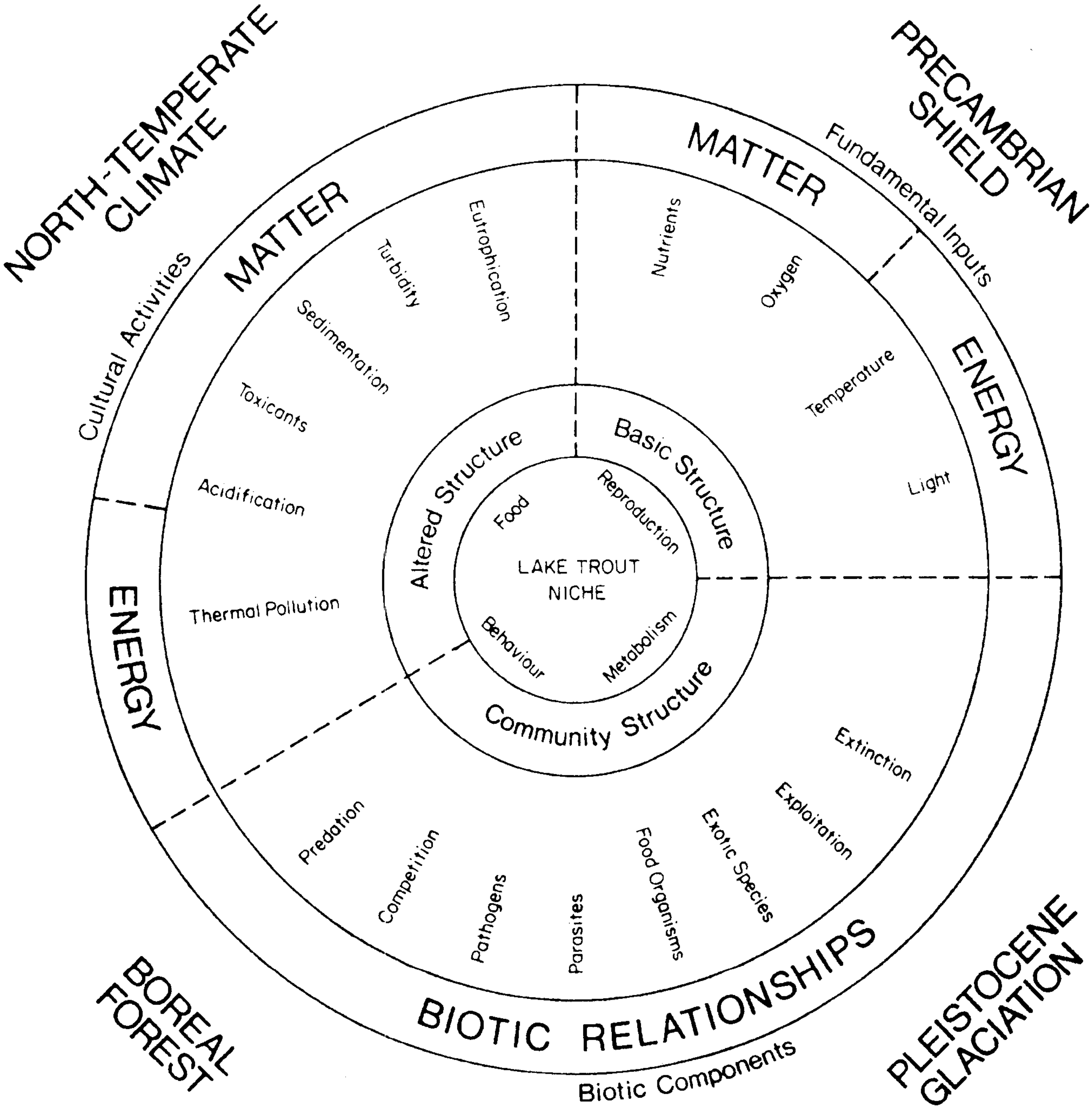


FIGURE 2 A two-dimensional representation of the "realized" lake trout niche pushed through time. The ecosystem attributes included in the diagram constrain the the fundamental dimensions of niche (that which is genotypically possible) into the realized form, which is a measure of environmental suitability.

To appreciate the complexity of identifying indicator species for virtual elimination of persistent toxic substances, one should consider the concept of the Fry-Hutchinson niche (Kerr and Ryder, 1977). Basically, each organism has an inherent, genetically determined scope for activity along many abiotic dimensions such as temperature, nutrients, oxygen, and light, as well as behavioural responses along several biotic axes such as reproduction, feeding or competition (Marshall *et al.* 1987); see Figure 2. However, the niche envelope usually shrinks along several dimensions in proportion to the degree of external constraints due to various natural, environmental controls. The realized niche is further constrained by cultural stresses superimposed onto a suite of natural background stresses (Marshall *et al.* 1987). Type I species are more sensitive to anthropogenic intrusions along one or more niche dimensions.

Toxicants are but one of a number of cultural influences that include habitat destruction, over exploitation, and introduction of exotic species. When Type I species such as the lake trout decline in an area, many factors must be considered as possible causes. Two workshops have been held by the Commission to review available evidence for cause-effect linkages between contaminants and declines in various species in the Great Lakes (Gilbertson 1989, Schneider and Campbell, 1991).

In the workshop, proposed surrogate or indicator species, both those identified by earlier Commission workshops, as well as new candidate species, were evaluated by: (1) considering the measurement scale the species, represents, e.g. keystone species in relation to the ecosystem; (2) considering the lifespan of the organism; (3) identifying which Great Lakes or habitats within the lakes are occupied by the species; (4) considering the sensitivity and the degree of specificity (sensitivity) to persistent toxic substances and which persistent toxic substances; (5) identifying the biochemical markers or biological effects which have been established in that species in response to persistent toxic substances; (6) considering the ease and estimated cost of measuring the specified effects for the indicator species; and (7) identifying research and data needs. Emphasis was placed on the 11 Critical Pollutants identified in Table 1. Using these criteria, the participants identified as many species as possible and grouped candidate species into five categories: mammals, birds, fish, reptiles, and invertebrates. A summary of the evaluation of each species as an indicator for virtual elimination of the presence and the injurious effects of persistent toxic substances, in a Great Lakes context, is presented below. More detailed consideration of developmental end points and biochemical markers for each species has been described above.

Mammals

Humans (*Homo sapiens*)

"The existence of toxic substances in the Great Lakes, which are used by hundreds of thousands of persons for recreational [and subsistence] fishing creates an opportunity and an obligation to scientifically address questions concerning the health of this ecosystem including its human population" (Humphrey 1988).

The studies of Rogan and Gladen (1985), Jacobson *et al.* (1985), Rogan *et al.* (1988) and Jacobson and Jacobson (1988) suggest that prenatal or breast milk exposure to PCBs can result in neurobehavioural deficits in human offspring. Although such manifestations of toxicity appear to be highly sensitive, they do not lend themselves to routine monitoring. The studies of Rogan *et al.* (1988) and Tetjak (1989) of accidentally and occupationally exposed communities provide considerable insight into what other physiological systems and types of measures might be most sensitive and/or specific to PCB toxicity in adults. Humans can be used as monitors of persistent toxic substances by measuring the pollutants in their blood, lipid, and breast milk but it is difficult to link adverse health impacts solely to persistent toxic substances.

Currently the EAGLE (Effects on Aboriginals of the Great Lakes Environment) Project, jointly funded by Health Canada and the Assembly of First Nations, is examining the effects of contaminants on health of aboriginals in over 60 communities in the Canadian portion of the Great Lakes basin. It is proposed that one phase of this project will include the collection of blood, urine and hair and the application of the caffeine breath test to a subgroup of the aboriginal population. This will allow a similar suite of biochemical markers to be measured in humans as is proposed for shoreline nesting bald eagles. Canadian and American studies are underway on cohorts of sports fishermen, and biochemical markers will be measured in some of these studies. The Ecosystem Health Work Group of the Commission's Science Advisory Board held a workshop in September 1992 which considered humans as bioindicators (EHWG in press). The report, "A Prescription for Healthy Great Lakes" (NWF/CIELAP, 1991) has also addressed human indicators as a measure of the state of well-being of the Great Lakes.

Mink (*Mustela vision*) and Otter (*Lutra canadensis*)

The most convincing reason for using the mink as an indicator of virtual elimination of persistent toxic substances is the species' very high sensitivity to reproductive effects caused by planar halogenated aromatic hydrocarbons (HCB, some PCBs, and TCDD) (Addison *et al.* 1991; Wren 1991). Studies in ranch mink suggest the mink is the freeliving mammal most sensitive to toxic substances such as PCBs and TCDD. Its diet provides an integrated exposure to contaminants in shoreline wetlands. Thriving mink populations in suitable Great Lakes shoreline wetlands would suggest that levels of these contaminants in their diet must be low. The chemical sensitivity of the otter is unconfirmed, but its largely piscivorous diet is more directly reflective of the nearshore aquatic environment.

However, "before a reliable operational biomonitoring program using mink (and otter) could be developed and employed, further research is needed to develop field survey techniques useful for the assessment of distribution, abundance and reproductive health of these species, and to determine the physiological and biochemical responses to chemical stressors which could be measured in free-living individuals" (Addison *et al.* 1991).

Ranch mink could also be used as a very sensitive bioassay for persistent toxic substances. Fish (carp) caught from various Great Lakes locations could be fed to captive mink and a variety of sensitive biological effects measured (Heaton *et al.* 1993).

Beluga Whale (*Delphinapterus leucas*)

The beluga whales resident in the St. Lawrence River estuary are also candidate indicators of virtual elimination of persistent toxic substances from the St. Lawrence River. The species has high public visibility and interest, is very sensitive to several of the persistent toxic substances targeted for virtual elimination, and is high on the food chain. Measures, beside the bioaccumulation of contaminants, include age structure of the population, incidence of tumors, various reproductive parameters, evidence of immune suppression and feminization of males, and presence of benzo-a-pyrene adducts in DNA of tissues. Beland *et al.* (1993) found levels of mercury, lead, PCBs, DDT, mirex, and other contaminants were all much higher in the St. Lawrence population than in the Arctic population that was used as a control. Their work suggests the beluga to be a viable indicator for the St. Lawrence River ecosystem. Because the St. Lawrence River beluga is classified as a threatened or endangered species, it cannot be sacrificed and only dead whales are available for study. Their size makes them difficult to handle or maintain in captivity. However, Arctic populations can serve as controls as can specimens existing in marine aquaria.

Birds

Bald Eagle (*Haliaeetus leucocephalus*)

The bald eagle was probably the first species that was widely affected by the introduction of DDT. According to the U.S. Fish and Wildlife Service, bald eagle numbers in the United States have recovered from a low of approximately 400 pairs nationwide in 1964 to 2,700 pairs in 1989. In the 1970s, when contaminant levels were the highest in the Great Lakes, the population along the Great Lakes shoreline and environs dwindled to 16 nesting pairs. Presently, the population is up to about 100 pairs, and the bald eagle is slowly beginning to reoccupy the Great Lakes coastal territories. However, reproduction is still impaired along Great Lakes shorelines of Michigan and Ohio and at inland sites accessible to runs of anadramous Great Lakes fish, and the incidence of congenital malformations may be increasing. Although suitable habitat exists, there are as yet no bald eagles nesting on the Lake Ontario shoreline.

It has been recommended that the Commission use the bald eagle as one of its "ecosystem objectives" as an indicator of overall habitat quality in the Great Lakes. As a mixed secondary-tertiary level predator, it feeds on large fish, waterfowl, colonial birds, turtles and muskrats. Like the human subpopulations which consume Great Lakes biota, this long-lived, slowly reproducing species is subject to the chronic effects of contaminant exposure. Tertiary predators provide an additional bioconcentration step and are therefore useful in detecting contaminants present at low levels. Bald eagles are highly sensitive to *p,p'*-DDE, dieldrin, PCBs, and possibly organic mercury (Bowerman *et al.* 1993, Colborn 1991). In any given bald eagle population, at least 50% of the breeding pairs must be successful in raising one or more young in any year, and the population as a whole must produce at least 0.7 fledged young per active nest to maintain population stability (Sprunt *et al.* 1973). Where suitable unoccupied nesting habitat exists, the successful re-establishment, normal reproduction, and population expansion of the bald eagle on Great Lakes shorelines can be used as a biological indicator of virtual elimination of persistent toxic substances. Its status as the national symbol of the United States and the high visibility of the bald eagle restoration program guarantee a high level of human interest and political support.

In order to monitor the levels and effects of persistent toxic substances in bald eagles, addled eggs must be salvaged and large young examined for gross anomalies and blood samples and feathers (where mercury accumulates) collected. Therefore the ease and cost of studying the bald eagle are considered moderate. Measures include eggshell thickness, the proportion of active territories fledging one or more young, the number of young fledged per active territory, adult turnover rate as measured by DNA fingerprinting or other technique, incidence of beak and feet deformities, MFO induction as measured by the caffeine breath test, and plasma retinol and thyroxine levels. These data are currently collected by investigators with the U.S. Fish and Wildlife Service and Michigan State University.

Herring Gull (*Larus argentatus*)

The herring gull has been extensively studied in the Great Lakes basin since the mid-1960s. This widespread species is a year-round resident in the basin (Gilman *et al.* 1977, Weseloh 1984). Both field studies (Fox *et al.* 1990) and a bioenergetics-based model suggest that Great Lakes herring gulls are opportunistic piscivores, and when forage fish are available, 80% of their diet is composed of fish. In 1974, the Canadian Wildlife Service initiated a surveillance project to monitor chemical contaminants and their effects in this species. Since its inception, the Great Lakes Herring Gull Monitoring Program has acquired one of the most complete and continuous databases in the world for contaminant residues and their associated effects. The biological measures utilized include egg viability (embryo mortality), chick growth and survival, incidence of deformities, and other components of GLEMEDS (Great Lakes Embryo Mortality Edema and Deformity Syndrome) (Gilbertson *et al.* 1991) and numbers. Monitoring using 28-day-old chicks will insure that observed levels and biochemical effects are the result of local stressors. The species is sufficiently well characterized that nutritional stress can be differentiated from toxic stress. Basinwide biomarker studies (Fox 1993) have been conducted for over 10 years and provide an existing historical database against which to measure progress toward virtual elimination. Studies of immune function and weight gain of chicks between 21 and 28 days of age have been successfully completed in this species and a study of DNA damage is underway.

Double-crested Cormorant (*Phalacrocorax auritus*)

The double-crested cormorant is a widespread obligate piscivore. However, it migrates from the Great Lakes basin for six months of the year. This species is more sensitive to DDE-induced eggshell thinning and PCB/TCDD-induced developmental abnormalities, particularly crossed bills, than is the herring gull. Recent outbreaks of Newcastle Disease, a highly pathogenic viral disease of poultry, suggests that this virus is endemic in Great Lakes cormorants and its presence will confound various biochemical marker responses. Bioeffects to be monitored in this species are eggshell thickness, and the incidence of bill abnormalities (Fox *et al.* 1991) and other abnormalities in chicks.

Forster's Tern (*Sterna forsteri*)

The distribution of the Forster's tern, a migratory piscivorous species which occupies eutrophic marshy habitats, is more-or-less limited in the Great Lakes basin to such areas in Green Bay, Saginaw Bay, and Lake St. Clair. The species is currently designated as threatened or endangered. Its limited distribution and its endangered status mitigate against its widespread use as an indicator species.

These birds nest in small colonies, usually on floating mats of vegetation, and are therefore rather difficult to study. However, the work of Kubiak *et al.* (1989) and Harris *et al.* (in press) illustrates their sensitivity to PCBs/TCDD. AHH is the only biochemical marker studied to date (Hoffman *et al.* 1987). Biological measures include hatching success, chick growth, and the incidence of deformities. Further work is necessary to establish standard methodology for assessment of reproductive measures.

Caspian Tern (*Sterna caspia*)

The Caspian tern is the largest tern in North America. This migratory species is a strict piscivore and nests in Lakes Ontario, Huron, Michigan, and Superior. It is considered threatened or endangered. The Caspian tern is a long-lived species and its populations within the Great Lakes basin have been the subject of large-scale, long-term banding efforts that suggest differential recruitment patterns between colonies in Canadian and United States waters (Ludwig 1979; L'Arrivee and Blokpoel, 1988), and a significant negative correlation between mean concentration of PCBs in the plasma of breeding adults and the proportion of young recruiting to that natal region has been reported by Mora *et al.* (1993). A more focused effort on this species would allow documentation and a better understanding of the population impacts of chronic exposure to Great Lakes contaminants in a long-lived fish-eating species. Recent studies suggest that, in terms of reproductive effects, this species is considerably more sensitive to PCBs than is the herring gull. Bioeffect measures include embryonic viability and the incidence of terata in dead eggs and abnormalities in chicks. Studies of immune function and weight gain of chicks between 21 and 28 days of age have also been successfully conducted in this species.

Black-crowned Night Heron (*Nycticorax nycticorax*)

The U.S. Fish and Wildlife Service has chosen this species as its estuarine and freshwater wetland sentinel species, and biochemical studies have been ongoing for several years at their Patuxent lab in Maryland. This species will be used as part of the nationwide BEST (Biomonitoring of Environmental Status and Trends) program. To date, the main factors being studied in the black-crowned night heron include reproductive success, eggshell thinning, MFO induction (AHH, EROD, BROD, and PROD) in pipping embryos and chicks (Custer *et al.* 1991; Hoffman *et al.* 1993), and a small amount of work on DNA damage. Other biochemical markers are under investigation and a joint Canadian Wildlife Service-U.S. Fish and Wildlife Service comparative study of biochemical marker responses of this species and herring gulls is planned.

Reptiles

Snapping Turtle (*Chelydra s. serpentina*)

The one reptile selected as a possible indicator was the snapping turtle. This long-lived omnivorous species commonly inhabits wetlands on the shorelines of the Great Lakes, and has a very limited home range throughout the year. These characteristics make the snapping turtle a good integrator of pollutant contamination within a local area (i.e. Areas of Concern). Hatching success of artificially incubated turtle eggs and the incidence of deformities in hatchling turtles have been measured at a number of sites within the basin (Bishop *et al.* 1991) and pilot biochemical studies have been initiated.

Fish

Lake Trout (*Salvelinus namaycush*)

According to Ryder and Edwards (1985), "the lake trout is the consummate integrator of oligotrophic biota by virtue of its function as the major aquatic terminal predator over most of the Great Lakes Basin" and is "the only indigenous terminal predator in the oligotrophic environment that satisfies the basic requirements of an ecosystem quality indicator." Historically, the lake trout was widely distributed in the main basins of Lakes Ontario, Huron, Michigan, Superior and the eastern and central basins of Lake Erie; approximately 95% of the surface waters of the basin. The lake trout is not only an excellent indicator of general ecosystem quality for oligotrophic systems, but its various responses also provide some diagnostic capability for specific stressors (Ryder and Edwards, 1985). The reproductive and early life history stages of the lake trout are especially vulnerable to environmental stresses and its failure to reproduce or to sustain progeny to recruitment into the fishery provides both an early-warning and a retrospective indication of system malfunction. The biological objective, utilizing properties of the lake trout, proposed by Ryder and Edwards has been incorporated into the 1987 revision of the Great Lakes Water Quality Agreement.

It is widely believed that, in the 1940s, lake trout populations in the Great Lakes were decimated by overfishing, sea lamprey parasitism, and degradation of prime spawning habitat. By the mid-1950s, the species was deemed extinct throughout the Great Lakes except for isolated populations in Lake Superior and two embayments in Georgian Bay. Despite the planting of over 150 million yearling-sized lake trout and successful re-establishment of adult lake trout in all the Great Lakes, little evidence of natural reproduction exists, particularly in Lakes Ontario, Michigan, and Erie. Stocked lake trout reach sexual maturity, produce viable gametes and successfully spawn; however only negligible numbers of their young survive.

Significant mortality has been documented during lake trout early development from these contaminated regions of the Great Lakes (Mac *et al.* 1985) and the presence of persistent organochlorine contaminants has been proposed to explain the early life stage mortality (Willford *et al. 1981*; Mac and Edsal, 1991). This mortality has been associated with reduced egg viability and swim-up mortality in sac fry. In the laboratory, Walker *et al.* (1991) have shown that "lake trout sac fry are more sensitive to the lethal effects of TCDD than any mammalian, avian, or [other] fish species investigated thus far". Based on egg TCDD dose, the LC_{50} for lake trout eggs is between 50-75 pg TCDD/g egg, regardless of whether exposure is via egg injection, the water, or the female (Walker *et al.* 1993). Following maternal deposition, egg TCDD concentrations >233 pg TCDD/g egg resulted in nonviable oocytes and failure of fertilization. In fertilized eggs, the manifestations of toxicity were identical to those observed in the field -- sac fry mortality associated with hemorrhages and yolk sac edema, resembling blue-sac disease. Maternally transferred PCBs reduce hatchability of eggs of Lake Michigan lake trout and egg survival is not related to swim-up survival (Mac and Edsal, 1991). PCB 105, but not other congeners, was highly correlated with egg mortality (Mac and Schwartz, 1993). Walker and Peterson (1991) found that PCB 105 did not cause egg or sac fry mortality in rainbow trout (*Oncorhynchus mykiss*) at egg doses as high as 6,970 ng/g whereas such effects were seen with 2-5 ng/g TCDD.

Hence, in the laboratory, researchers have illustrated a cause-effect linkage between contaminants and reproductive failure in lake trout, suggesting that persistent organic contaminants are a factor in the inability of lake trout to maintain self-sustaining populations in some of the Great Lakes (Mac and Edsal, 1991). Binder and Lech (1984) found hepatic AAH activities in lake trout swim-up fry from Lake Michigan and Green Bay to be markedly elevated compared to identical genetic hatchery stock when eggs were cultured under the same hatchery conditions. These differences parallel relative differences in contamination, measured as PCBs. Cook *et al.* (1993) provide a sound epidemiological argument suggesting that TCDD and TCDD-like polyhalogenated aromatic hydrocarbons played a major role in the reproductive failure of lake trout in Lake Ontario. Recently, Delorme *et al.* (1993) at the Freshwater Institute have treated a tagged subsample of a viable reproducing population of lake trout inhabiting a small isolated lake in northwestern Ontario with a single injection of 2,3,4,7,8-PCDF. These fish have been monitored for four successive years. They show sustained EROD induction and decreased retinoid storage, reproductive and demographic differences suggesting that this methodology would allow the definitive testing of this hypothesis. Such a field study should be encouraged and based on the findings of Walker *et al.*, using TCDD.

Brown *et al.* (1993) have treated immature lake trout with a single oral dose of PCB 126. Liver size and hepatic EROD activity were elevated in a dose-dependent manner. They concluded that "although fish growth or condition were unaffected, both altered retinoid homeostasis and elevated hepatic EROD activity were sensitive indicators of coplanar PCB exposure in lake trout".

The ease and the cost of using lake trout as an ecological indicator were thought to be moderate. Emphasis should be placed on monitoring for successful reproduction and fry survival to one year in Lakes Michigan, Ontario, and Erie. Biochemical measures such as MFO induction (Binder and Lech, 1984; Luxon *et al.* 1987; Hodson *et al.* 1989) and retinoid stores can provide further information on the exposure of the adult populations to persistent toxic substances.

Walleye (*Stizostedion vitreum*)

Like the lake trout, the walleye is a carnivore, feeding on other fish. According to Ryder and Edwards (1985) "abundant, reproducing stocks of walleyes, free of contaminant burdens, will be indicative of high quality mesotrophic environments, particularly those of the nearshore littoral and limnetic zones, river deltas and major tributary systems" of the Great Lakes. Historically, prime walleye habitat included such areas as the Bay of Quinte, most of Lake Erie but particularly the western basin, Lake St. Clair, Saginaw Bay, Georgian Bay, Green Bay, the North Channel, parts of Nipigon Bay and Black Bay, and most connecting channels.

Walleye are still present in these areas and are prized by subsistence, commercial, and sport fishermen alike. Walleye populations in western Lake Erie were affected in the 1960s by overfishing and mercury contamination, as reflected in the young-of-the-year index, growth, and population age distribution (Hatch *et al.* 1987). There are considerable data on levels of persistent toxic substances, particularly mercury, in the tissues of walleye from Lake Erie, Lake St. Clair, and the St. Clair and Detroit Rivers.

There are few, if any, data on biomarkers in this species and this was flagged as a research need by the workshop participants. There is no obvious reason why those measures applied in lake trout could not be measured in walleye. The use of an index of annual recruitment such as the young-of-the-year index, based on a catch per hour with a small trawl fished biweekly at fixed sampling sites mid-June through October, age-size distribution, and a measure of catch per unit effort would provide insight into the status of the population.

White Sucker (*Catostomus commersoni*)

Munkittrick and Dixon (1989a) "believe that monitoring populations of white sucker has the potential to reduce the time lag between lower level responses and detection of changes in the fish populations. White sucker have direct exposure to contaminated sediments and are dependent on invertebrate species for food throughout their life cycle." The underlying principle of their framework for determining the effects of chronic exposure to contaminants on fish populations is "that a population of fish found to be growing, reproducing, and surviving within the limits of a comparable reference population will be considered free from detrimental contaminant-exposure effects." They believe monitoring white sucker populations may be the least expensive and most attractive method for obtaining first indications of impact in toxicant-sensitive or high-risk areas. However, individual or population responses which do not affect growth, survivorship, or reproduction will not be detected and may be obscured by migration or fishing pressure. A large number of biomarkers have been successfully measured in this species including ALAD, various MFO activities, clinical biochemistry, thyroid and steroid hormones, measures of energy stores and bioeffects such as growth rate, secondary sex characteristics, fecundity, and age at maturity.

A bottom-feeder, the white sucker is abundant and widespread in the Great Lakes, and frequently exposed to contaminated sediments. This relatively sedentary species tends to home to and spawn in small streams in spring, where large numbers can be captured easily. The white sucker has been shown to be affected by PAHs, lead and other metals, contaminants in bleach kraft pulp mill effluents, and a variety of other chemicals. They are a direct link between the sediments and the animals highest in the food chain, eagles and humans. Several authors have suggested that wild fish may be useful sentinels for aquatic pollution by carcinogenic chemicals, particularly PAHs, and may provide a direct epizootiological indicator of excessive pollution or successful remediation. The white sucker is known to have a low incidence of hepatobiliary and epidermal neoplasms, which increases with contact with contaminated sediments (Hayes *et al.* 1990, Government of Canada 1991).

Brown Bullhead (*Ictalurus nebulosus*)

The brown bullhead, a bottom-feeding omnivore, is abundant and widespread in the Great Lakes. It prefers to live in quiet, warm, shallow, and weedy waters, where it is a good local indicator for PAH-contaminated sediments. Baumann *et al.* (1990) and Baumann (1992) compared the incidence of biliary carcinoma and hepatocellular carcinoma and age structure in brown bullheads >25 cm in length from two populations and found they were related to changes in PAH levels in the sediments. PAH metabolites can be measured in their bile (Maccubbin *et al.* 1988) and their liver/body mass ratio determined (Fabacher and Baumann, 1985).

Phytoplankton, Zooplankton, and Benthic Invertebrates

Much of the biological work on the effects of pollution and on the search for bioindicators during this century has been focused on phytoplankton and zooplankton assemblages and various benthic invertebrate communities. Water quality criteria have been successfully developed for conventional pollutants using laboratory cultures of the various organisms and their responses to pollutants in toxicity tests. They have also been used extensively as bioassay organisms (*Selenastrum, Chlorella, Daphnia, Ceriodaphnia, Diporeia*, and *Hexagenia spp.*) to study the effects of individual contaminants or to test effluents when the specific identity of the polluting substances was not necessarily known. Unfortunately, there are few scientifically defensible cases of specific effects in phytoplankton or invertebrate species, assemblages, or communities which have been associated with the persistent toxic substances targeted for virtual elimination (Cook *et al.* 1993). **Although these organisms are of fundamental importance in monitoring ecosystem function and integrity, they are of limited value in tracking virtual elimination of persistent toxic substances.**

Phytoplankton form the most important group of primary producers in aquatic food webs. In general, phytoplankton are sensitive to toxicants and, because of their short generation time and rapid physiological responses, it is possible to detect deleterious effects of toxicants in relatively short periods of time (Sicko-Goad and Stoermer, 1988).

Phytoplankton have been experimentally shown to be sensitive to both metals and organic chemicals, including several of the persistent toxic substances of concern. However, their physiological responses, which include reduction or inhibition of cell division, senescence or dormancy, destruction of membranes and membranous organelles, and reduction in photosynthesis are not compound- or group-specific. Phytoplankton abundance and species composition are integrative responses to perturbations in the lake ecosystem and can be used to trace long-term changes in lakes (Munawar and Munawar, 1982).

The zooplankton community responds particularly to changes in food resources and selective predation by changes in community structure. Zooplankton abundance in the Great Lakes varies as a function of trophic status. Thus, zooplankton have value as indicators of water quality and the structure of biotic communities. In addition, zooplankton can potentially affect cycling of contaminants through the food web via metabolism, transport, and biomagnification. In zooplankton, reproductive measures appear more sensitive than measures based on mortality and physiological function. Although there are good laboratory data describing effects of contaminants on zooplankton reproduction, grazing rates, biomass and growth, field studies in the Great Lakes have been unable to differentiate effects caused by exposure to chemicals from the effects of eutrophication and fish predation (Evans and McNaught, 1988).

Benthic organisms play an integral role in the effects, fate, and cycling of contaminants throughout the rest of the aquatic ecosystem. Concentrations of contaminants in the benthos provide an indication of the bioavailablity of a particular contaminant. Whether or not contaminants have altered the structure of Great Lakes benthic communities is difficult to assess. Unless concentrations of a particular contaminant are high and the resulting impact on the benthos is quite dramatic, changes in community structure resulting from chemical contaminants cannot be readily distinguished from other perturbations such as organic pollution, cultural eutrophication, or natural and human-induced ecological changes (Nalpea and Landrum, 1988). However, Reynoldson and Zarull (1989) used an integrated strategy for the assessment and delineation of contaminated sediments in the Detroit River which demonstrated linkages between levels of contaminants (including HCB, mercury, and lead) and benthic community responses.

Midge (*Chironomidae*)

The family Chironomidae is ubiquitous and represents the single largest family of aquatic insects. Chironomid communities are concentrated in the near-surface sediments. Their life cycle is short compared to other biological indicators -- one to three generations per year. Hence, the morphological responses of chironomid larvae should be a reasonably accurate reflection of conditions in the sediments at the time of sampling (Warwick 1988). Morphological anomalies may range from antennal deformities or mildly abnormal mouth parts to the grotesque thickening and fusing of all body structures.

The remains of chironomid larvae, particularly the head capsule, preserve well in most sediments. Palaeoanalysis of the frequency and severity of deformities in the remains of chironomid larvae present in the yearly increment of sediment provide a temporal measure of ambient conditions. Warwick concludes that "evidence for a cause-effect relationship between morphological deformities and environmental contaminants remains largely circumstantial -- deformities increase in populations exposed to industrial wastes and/or agricultural residues but not in populations exposed to domestic wastes." Experimental evidence indicates that dieldrin and DDE can induce deformities in chironomid cultures similar to those observed in contaminated ecosystems.

Although chironomid larvae are easily collected, the cost of assessment is high. Specifically, slide preparation, necessary to properly study deformities, is time consuming.

Mayfly (*Hexagena sp.)*

Reynoldson *et al.* (1989) reviewed historical changes in the benthic invertebrate communities of three Great Lakes mesotrophic systems: western Lake Erie, Saginaw Bay, and Green Bay. In all three systems, there was a shift in the 1950s from a community dominated by the burrowing mayfly *Hexagenia limbata* to a community dominated by chironomids or oligochaetes. They conclude that "although contaminants may be a contributing factor, the weight of evidence suggests oxygen depletion, a result of increased nutrient loading, as the major factor in changes in benthic community structure." These changes reflect trophic state. Reynoldson *et al.* (1989) believe that benthic community structure would be an appropriate indicator of mesotrophic conditions, and that a desirable objective would be an open lake benthic invertebrate community where *Hexagenia* is the dominant organism. Other investigators have used survival and behaviour of *Hexagenia* as a bioassay of sediments (Prater and Anderson, 1977) and indicator organisms in which to measure levels of persistent toxic substances (Clements and Kawatski, 1984).

5. CONCLUSIONS AND RECOMMENDATIONS

COMPONENTS OF A VIRTUAL ELIMINATION BIOMONITORING PROGRAM

One goal of the workshop was to provide guidance about the need for, and the use of bioindicators in relation to virtual elimination of persistent toxic substances. The material presented earlier identifies the type of data and information required in order to measure injury (or the absence thereof) caused by the presence of persistent toxic substances in the Great Lakes Basin Ecosystem. In particular, this report identifies biochemical markers, biological effects, indicator species, and the criteria for their evaluation and selection for use. In sum, the criteria included consideration of scale (organizational, trophic, temporal, and spatial), specificity (sensitivity) to persistent toxic substances (as opposed to other confounding factors), applicability of a biomeasurement to a particular species, and the ease and cost associated with sample collection and analysis.

Based upon the presentations to, and deliberations at the workshop, a program to document trends in the incidence and severity of fish and wildlife injury as a measure of virtual elimination of persistent toxic substances in the Great Lakes Basin Ecosystem should include the bioindicators listed in Table 4. A number of these bioindicators are noted as "to be developed or adapted, and validated in this species," and should be tested in the laboratory and/or field.

The proposed scheme utilizes different species in different environments. The species recommended for use basinwide were chosen on the basis of their sensitivity (bald eagle), basinwide distribution (herring gull, cormorant, and white sucker), and established use as sentinel species by one or mor agencies (herring gull, night heron, white sucker). Where possible, parallel measures are recommended in a fish and bird species. Properties of the biology of lake trout, bald eagle, walleye, and *Hexagenia* have already been recommended to the Commission as "ecosystem objectives" for the environments in which we propose these species be used. The suite of species proposed for use in Areas of Concern were chosen because they have been well studied in one or more Areas of Concern (Caspian tern, snapping turtle, and brown bullhead) or are sensitive to particular chemical-related concerns in these areas (brown bullhead, *Hexagenia*, and the benthic invertebrate community). Measures suggested for the bald eagle and lake trout will have considerable commonality with those made in populations of human fish-eaters in the Great Lakes basin. *Although necessary, we recognize that the species and measures proposed in Table 4 may not be sufficient to track virtual elimination, and that other components may need to be developed and implemented, as discussed below.*

This information is necessary to track progress toward, and achievement of the virtual elimination goal of the Great Lakes Water Quality Agreement. It is recommended that:

1. **The Parties consider incorporation of the bioindicators presented in Table 4 into the Great Lakes Water Quality Agreement and implement basinwide biomonitoring programs that will detect injury and increase understanding of those injuries currently documented in Great Lakes biota.**

2. **The Commission use bioindicator data and information to evaluate progress toward, and achievement of the Agreement's virtual elimination goal.**

To the extent possible, the information should be based on field data, which should be consistent with laboratory-generated data and cohere with theory. All data must be acquired using standard methods and procedures.

DATA AND PROGRAM NEEDS

To track progress toward, and achievement and maintenance of the Agreement's virtual elimination goal, long-term biomonitoring programs must be initiated. These must cover the entire food web, the Great Lakes basin, and ensure acquisition of a long-term data base. These programs must focus on measurement of injury in wild populations that has been attributed to persistent toxic substances.

McCarthy (1990) concluded that "core capabilities for measuring a fairly wide array of candidate biomarkers do exist in federal agencies, national laboratories and universities and sufficient experience exists for making rational choices about selection and sampling of animal species. *The primary impediments to major progress in applying this approach to environmental monitoring is the lack of a unifying mandate and the need for stable, long-term funding.*"

TABLE 4. ***Proposed Scheme for Monitoring Virtual Elimination of Injury and Impairments Associated with Persistent Toxic Substances in Great Lakes Biota***

Area and Indicator Species	**Population Character-istics**	**Repro-ductive Success**	**Chick Growth**	**Congenital Anomalies**	**Eggshell Thinning**	**Tumors**	**EROD/AHH or Caffeine Breath Test**	**PROD BROD**	**Vitamin A Stores**	**Porphyrin Pattern**	**Plasma Thyroxine**	**DNA Damage**	**Plasma ALAD**
BASINWIDE													
Bald eagle	Y	Y	Y	Y	Y	N	CBT	N	Plasma	N	Y	N	(Y)
Herring gull or Black-crowned night heron	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	N	N
Double-crested cormorant	Y	N	N	Y	Y	N	N	N	N	N	N	N	N
White sucker	Y	Y	N	N	N	Y	Y	[Y]	[Y]	N	N	[Y]	Y
OLIGOTROPHIC													
Lake trout	Y	Y	N	N	N	N	Y	N	Y	N	Y	N	N
SHORELINE WETLANDS													
Mink	[Y]	[Y]	N	N	N	N	N	N	N	N	N	N	N
Snapping turtle	Y	Y	N	Y	N	N	N	N	N	N	N	[Y]	N
MESOTROPHIC													
Forster's Tern	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N
Walleye	Y	Y	N	N	N	N	[Y]	N	[Y]	N	Y	N	N
Hexagenia	Y	N	N	N	N	N	N	N	N	N	N	N	N
ST. LAWRENCE RIVER													
Beluga	Y	Y	N	Y	N	N	N	N	N	Y	N		
AREAS OF CONCERN													
Caspian Tern	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N
Snapping turtle	Y	Y	N	Y	N	N	N	N	N	N	N	[Y]	N
Brown bullhead	Y	N	N	N	N	Y	N	N	N	N	N	[Y]	[Y]
Hexagenia	Y	N	N	N	N	N	N	N	N	N	N	N	N
Benthic community	Y	N	N	N	N	N	N	N	N	N	N	N	N

LEGEND
Y = Yes
N = No
(Y) = ALAD in eagles would be a good monitor for all forms of lead in food chain
[ ] = Biomarker to be developed or adapted, and validated in this species

The governments have initiated and/or are operating a number of surveillance and monitoring programs which have a potential bioindicator orientation and which *could* serve as vehicles for the provision of information to track virtual elimination of persistent toxic substances and elimination of toxic effects. These include EMAP (Environmental Monitoring and Assessment Program) (U.S. EPA), National Status and Trends Program (NOAA), National Water Quality Assessment Program (USGS), BEST (Biomonitoring of Environmental Status and Trends) Program, (U.S. FWS), CWS's ongoing program on fish-eating birds and snapping turtles, Canada DFO's monitoring of lake trout and studies of white suckers, and EAGLE (Health Canada). A number of these programs deliver the requisite data on wild species. Others focus on measurement of contaminant levels. However, since some of these programs are in their formative stages, there is opportunity to redirect them toward the measurement of injury. However, there is no regulatory mandate to develop these data, perhaps because the need has not been recognized, nor the apparent conflicts with current regulatory approaches assessed.

It is recommended that:

3. **The Parties review and reorient their surveillance and monitoring programs to ensure documentation of injury and impairment in wild populations and to ensure the acquisition of a long-term data base which covers the whole basin.**

If these programs are to be used as vehicles for delivery of requisite data and information, they must be coordinated to ensure that they are complementary and deliver the information required, for use in a virtual elimination context.

No one program can meet all the biomonitoring needs but, taken together and properly augmented, these programs could provide the necessary framework. It is essential that governments provide long-term commitment and funding for monitoring those species and bioindicators that are deemed necessary and scientifically justified.

There is also a need to interpret and present these data in terms understandable by the general public and by environmental and resource managers. Proper interpretation and clear presentation will help ensure proper use to develop policy and influence decision making. As a component of interpretation, benchmark or reference data are required as a basis against which to measure change; this approach may include data from areas relatively unaffected by persistent toxic substances, so that the goal of absence of injury can be quantified.

Also, to ensure protection of humans from the effects of persistent toxic substances, there is a need to establish a link between injury observed in wildlife and measures that reflect the well being of human fish-eaters.

In addition to developing and implementing programs to generate data now and in the future, there is a need to archive samples in a systematic manner. Archiving or specimen banking is essential to provide historic samples for retrospective analysis to determine trends and document changes, when additional persistent toxic substances come under scrutiny or if new test procedures are developed. It is recommended that:

4. **The Parties review and upgrade specimen banking programs, particularly in the United States. Facilities should be equipped and efforts made to archive suitable tissues for BOTH chemical residue and biochemical measurements.**

RESEARCH NEEDS

One goal of the workshop was to provide advice about research to be undertaken to close information gaps, through development of additional biochemical markers and appropriate test protocols, for application to requisite biological species.

Further research is needed on the chemicals and other factors responsible for changes in the biomarkers recommended in this report. Much more research is needed to define biomarker variability and to characterize homeostasis, compensation, and injury and to understand and eliminate organismal, environmental, and methodological components of this variation. Further field validations of these biomarkers are needed, preferably using different species and experimental approaches. Every opportunity should be taken to document associations between biomarker responses and other ecologically relevant effects at the population level, particularly those affecting growth, survival, and reproduction. To provide valid, unambiguous information, the associated sample collection, analysis, and data interpretation procedures must be standardized and quality assured. In recognition that budgets and funding are constrained, sample analysis should be cost effective and the data itself easily acquired and interpreted.

Developmental work is required for biochemical markers whose validity has been demonstrated for certain fish and wildlife species, but for which application to, and validation for other species may be desirable (e.g. Vitamin A storage and porphyria in fish; the caffeine breath test in eaglets, and DNA adducts and damage in birds and mammals). There are gaps in the suite of biochemical markers to measure the type and extent of injury. Functional biochemical markers and test procedures need to be developed and applied to detect, for example, immune toxicity (TCDD, PCBs, mercury, lead), neurotoxicity (e.g. lead, mercury, dieldrin, DDT, and some PCBs), and nephrotoxicity (mercury, cadmium).

Some fish and wildlife species have been identified as potentially useful sentinels in which to measure injury due to persistent toxic substances. However, the utility of these species must be validated, e.g. walleye. Human beings present a special case: is it possible to identify biomarkers that are specifically affected by persistent toxic substances that can be used in surveys of human fish-eaters? At present such indicators exist for cytochrome P450-1A1 inducers, lead and mercury.

It is recommended that:

5. **The Parties provide funding for such research in their reoriented surveillance and monitoring programs and that funding sources such as the Great Lakes Protection Fund, Wildlife Toxicology Fund, Natural Science and Engineering Research Council, National Science Foundation, and Great Lakes University Research Fund consider such research for funding.**

Most currently available bioindicators are applied to indicator species at or near the top of the food chain. There is a lack of biochemical markers for species which occupy lower trophic levels and which could provide an early indication of injury. To provide full coverage of the food web, there is a need to investigate the feasibility of developing and applying bioindicators to biota such as bacteria, phytoplankton, zooplankton, and molluscs.

A number of cause-effect associations involving persistent toxic substances and injury have been developed in recent years. This is a rather lengthy process. Researchers develop the relevant science and disseminate the resulting data and information. Consensus on cause-effect takes time to develop within the scientific community. There is no established institutional mechanism, and no person or entity has assumed responsibility to synthesize and assess scientific data and information from diverse studies and to develop cause-effect linkages. Without such a determination, the science cannot fully influence policy makers. The Commission, in its binational collaborative capacity, may be an appropriate institution to fulfil this role.

It is recommended that:

6. **The Commission assist the Parties by undertaking to develop a framework within which the scientific community can examine injury of Great Lakes biota to establish linkages between observed biological effects and causes, including persistent toxic substances, and to ensure that this information reaches policy makers in a timely fashion.**

The population declines and extinction of the lake trout which began in the 1940s have had a significant impact on the fishery of the Great Lakes region. The injury to this magnificent resource was the first of many to be documented in Great Lakes biota, and is probably the best studied. Today, despite the re-establishment of adult lake trout in all the Great Lakes, there is little evidence of natural reproduction, particularly in Lakes Ontario, Michigan, and Erie. Stocked lake trout reach sexual maturity, produce viable gametes and successfully spawn; however only negligible numbers of their young survive. Strong epidemiological arguments and laboratory studies suggest that polyhalogenated aromatic hydrocarbons, particularly TCDD and TCDD-like congeners are responsible for the reproductive impairment.

The lake trout has been incorporated into the Agreement as a general "ecosystem objective." However, laboratory studies suggest that the sac-fry of this indigenous predator are more sensitive to the lethal effects of TCDD than any other vertebrate species investigated thus far. To transform the lake trout from a "general ecosystem objective" to a very sensitive "specific ecosystem objective for the virtual elimination of dioxin-like toxicity," it is necessary to clarify the role of these persistent toxic substances in the reproductive impairment of free-living lake trout.

It is recommended that:

7. **The Parties, in collaboration with the Habitat Advisory Board of the Great Lakes Fishery Commission, experimentally test the hypothesis that TCDD and TCDD-like polyhalogenated aromatic hydrocarbon congeners play a major role in the reproductive impairment of lake trout in the Great Lakes, using a subsample of a viable reproducing population inhabiting a small, isolated lake.**

If, in the search for better tools to understand nature, unequivocal biochemical markers for lower trophic levels are not feasible, then alternative approaches such as monitoring for the restoration of healthy, ecologically desirable phytoplankton and zooplankton communities is desirable. Standard *in vitro* bioassays may serve as appropriate surrogates to provide early warning of possible impact on species at all levels in the food web. Bioassays could also serve as a preliminary screen. These tests (e.g. Microtox) are already standardized and include organisms from many different trophic levels (including fathead minnow, *Ceriodaphnia*, algae, and bacteria). There should also be encouragement for the development of reliable and predictive *in vitro* tests so that the use of live animals can be reduced.

The field of biomonitoring and the use of bioindicators is evolving rapidly. This workshop is just one exercise contributing to this effort. The Commission's Council of Great Lakes Research Managers may be an appropriate entity:

- To track and periodically review ongoing developments.
- To further scope out/develop research needs.
- To communicate these research needs to the research community.
- To update the suite of indicators and indicator species.

The Council's inventory of Great Lakes research should be consulted to ascertain whether relevant work is presently under way (CGLRM 1993).

REFERENCES

Addison, E.M., G.A. Fox, and M. Gilbertson. 1991. Proceedings of the expert consultation meeting on mink and otter. Great Lakes Science Advisory Board's Ecological Committee, Report to the International Joint Commission, Windsor, Ontario. 30 pp.

Baumann, P.C. 1992. Reduced PAH causes decreased liver tumors and increased lifespan in Black River bullheads. Page 156. In: Abstracts of 13th Annual Meeting of Soc. Environ. Toxicol. Chem., Cincinnati.

Baumann, P.C., J.C. Harshbarger and K.J. Hartman. 1990. Relationship between liver tumors and age in brown bullhead populations from two Lake Erie tributaries. Sci. Total. Environ. 94:71-87.

Bayne, B.L., D.A. Brown, K. Burns, D.R. Dixon, A. Ivanovichi, D. R. Livingstone, D.M. Lowe, M.N. Moore, A.R.D. Stebbings and J. Widdows. 1985. *The Effects of Stress on Marine Animals.* Praeger Publ., New York. p. 384.

Beland, P., S. De Guise, C. Girard, A. Lagase, D. Martineau, R. Michaud, D. Muir, R. Norstrom, E. Pelletier, S. Ray, and L. Shugart. 1993. Toxic Compounds and Health and Reproductive Effects in St. Lawrence Beluga Whales. J. Great Lakes Res. 19, No. 4.

Best, D.A., M. Gilbertson and H. Hudson (eds.) 1990. Proceedings of the expert consultation meeting on bald eagles. Great Lakes Science Advisory Board's Ecological Committee, Report to the International Joint Commission, Windsor, Ontario. 33 pp.

Binder, R.L. and J.J. Lech. 1984. Xenobiotics in gametes of Lake Michigan lake trout (*Salvelinus namaycush*) induce hepatic monoxygenase activity in their offspring. Fund. Appl. Tox. 4:1042-1054.

Birnbaum, L.S. (in press). The mechanism of dioxin toxicity: relationship to risk assessment. Environ. Health Perspect.

Bishop, C.A., R.J. Brooks, J.H. Carey, P. Ng, R.J. Norstrom, and D.R.S. Lean. 1991. The case for a case-effect linkage between environmental contamination and development in eggs of the common snapping turtle (*Chelydra s. serpentina*) from Ontario, Canada. J. Toxicol. Environ. Health 33:521-547.

Bowerman, W.B., D. Best, T. Kubiak, S. Postupalsky, and D. Tillit. 1993. Bald eagle reproductive impairment around the Great Lakes: Association with organochlorine contamination. J. Great Lakes Res. 19, No. 4.

Brett, J.R. 1958. Implications and assessments of environmental stress. Pages 69-83 In: P.A. Larkin (ed.) *Investigations of Fish-power Problems.* Univ. Brit. Col. Press, Vancouver.

Brouwer, A. and J.K. Van den Berg. 1984. Early and differential decrease in natural retinoid levels in C57B1/Rij and DBA/2 mice by 3,4,3',4'-tetrachlorobiphenyl. Toxicol. Appl. Pharmacol. 73:204-209.

Brown, S.B., V.P. Palace, D.A. Metner, J.F. Klaverkamp and W.L. Lockhart. 1993. Effect of coplanar 3,3'4,4',5-pentachlorobiphenyl on retinoids, tocopherol and hepatic enzyme levels in lake trout (*Salvelinus namaycush*). Page 11 In: Abstracts of 36th Conference of Internat. Assoc. Great Lakes Res., De Pere, Wisconsin.

Clements, J.R. and J.A. Kawatski. 1984. Occurrence of polychlorinated biphenyls (PCBs) in adult mayflies (*Hexagenia bilineata*) of the upper Mississippi River. J. Freshwater Biol. 2:611-614.

Colburn, T. 1991. Epidemiology of Great Lakes bald eagles. J. Toxicol. Environ. Health 33:395-453.

Cook, P.M., R.J. Russell, R.L. Spehar, S.P. Bradbury and G.T. Ankley. 1993. *Interim Report on Data and Methods for Assessment of 2,3,7,8-Tetrachlorodibenzo-p-dioxin Risks to Aquatic Life and Associated Wildlife.* EPA/600/R-93/055. U.S. Environmental Protection Agency, Office of Research and Development, Duluth, Minnesota.

Council of Great Lakes Research Managers. 1991. A Proposed Framework for Developing Indicators of Ecosystem Health for the Great Lakes Region. International Joint Commission, Windsor, Ontario. 47 pp.

Council of Great Lakes Research Managers. 1993. Great Lakes-St. Lawrence Research Inventory. Report to the International Joint Commission. Windsor, Ontario.

Custer, T.W. B.A. Rattner, H.M. Ohlendorf and M.J. Melancon. Herons and egrets as proposed indicators of estuarine contamination in the United States. 1991. Acta 20th Congressus Internationalis Ornithologici: 2474-2478.

Delorme, P.D., W.L. Lockhart, D.C.G. Muir and F.J. Ward. 1993. Sustained induction of hepatic EROD activity in feral lake trout and white suckers given single intraperitoneal injections of [14C]-2,3,4,7,8-pentachlorodibenzo-furan. Page 112 In: Abstracts of 36th Conference of Internat. Assoc. Great Lakes Res., De Pere, Wisconsin.

Depledge, M. 1989. The rational basis for detection of the early effects of marine pollutants using physiological indicators. Ambio 18:301-302.

Drash, G.A., D. Walser and J. Kosters. 1987. The urban pigeon (*Columba liva, forma urbana*) - a biomonitor for the lead burden of the environment. Environ. Monit. Assess. 9:223-232.

Ecosystem Health Workgroup (in press). Our Community, Our Health: Dialogue Between Science and Community. A report on a workshop held September 14-15, 1992. Great Lakes Science Advisory Board, International Joint Commission, Windsor, Ontario.

Edwards, C.J. and R.A. Ryder. 1990. Biological surrogates of mesotrophic ecosystem health in the Laurentian Great Lakes. Report to the Great Lakes Science Advisory Board, International Joint Commission, Windsor, Ontario. 69 pp.

Evans, M.S. and D.C. McNaught. 1988. The effect of toxic substances on zooplankton populations: a Great Lakes perspective. Pages 53-76 In: M.S. Evans (ed.), *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus.* J. Wiley and Sons, Toronto.

Fabacher, D.L. and P.C. Baumann. 1985. Enlarged livers and hepatic microsomal mixed-function oxidase components in tumor-bearing brown bullheads from a chemically contaminated river. Environ. Toxicol. Chem. 4:703-710.

Fox, G.A. 1991. Practical causal inference for ecoepidemiologists. J. Toxicol. Environ. Health 33:359-373.

Fox, G.A. 1993. What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. J. Great Lakes Research 19:722-736.

Fox, G.A., L.J. Allan, D.V. Weseloh, and P. Mineau. 1990. The diet of herring gulls during the nesting period in Canadian waters of the Great Lakes. Can. J. Zool. 68:1075-1085.

Fox, G.A., B. Collins, E. Hayakawa, D.V. Weseloh, J.P. Ludwig, T.J. Kubiak and T.C. Erdman. 1991. Reproductive outcomes in colonial fish-eating birds: a biomarker for developmental toxicants in Great Lakes food chains. II. Spatial variation in the occurrence and prevalence of bill defects in young double-crested cormorants in the Great Lakes, 1979-1987. J. Great Lakes Res. 17:158-167.

Fox, G.A., M. Gilbertson, A.P. Gilman, and T.J. Kubiak. 1991. Editorial. A rationale for the use of colonial fish-eating birds to monitor the presence of developmental toxicants in Great Lakes fish. J. Great Lakes Res. 17:151-152.

Fox, G.A., S.W. Kennedy, R.J. Norstrom and D.C. Wigfield. 1988. Porphyria in herring gulls: a biochemical response to chemical contamination of Great Lakes food chains. Environ. Toxicol. Chem. 7:831-839.

Gilbertson, M. (ed.). 1989. Proceedings of the Workshop on Cause-Effect Linkages, 28-30 March 1989, Chicago. Council of Great Lakes Research Managers, International Joint Commission, Windsor, Ontario. 45 pp.

Gilbertson, M., T. Kubiak, J. Ludwig, and G. Fox. 1991. Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: Similarity to chick-edema disease. J. Toxicol. Environ. Health 33:455-520.

Gilman, A.P., G.A. Fox, D.B. Peakall, S.M. Teeple, T.R. Carroll, and G.T. Hames. 1977. Reproductive parameters and egg contaminant levels in Great Lakes herring gulls. J. Wildlife Manage. 41:458-468.

Government of Canada. 1991. *Toxic Chemicals in the Great Lakes and Associated Effects.* Vol. II. *Effects.* Supply and Services Canada, Ottawa.

Harris, H.J., T.C. Erdman, G.T. Ankley and K.B. Lodge. (in press). Measures of reproductive success and PCB residues in eggs and chicks of Forster's tern on Green Bay, Lake Michigan - 1988. Arch. Environ. Contam. Toxicol. (in press)

Hatch, R.W., S.J. Nepszy, K.M. Muth and C.T. Baker. 1987. Dynamics of the recovery of the western Lake Erie walleye (*Stizostedion v. vitreum*) stock. Can. J. Fish. Aquat. Sci. (Suppl. 2):15-22.

Hatch, T. 1962. Changing objectives in occupational health. Am. Ind. Hyg. Assoc. J. 23:1-7.

Haux, C., A. Larsson, G. Lithner and M.-L. Strobeck. 1986. A field study of physiological effects on fish in lead-contaminated lakes. Environ. Toxicol. Chem. 5:283-288.

Hayes, M.A., I.R. Smith, T.H. Rushmore, T.L. Crane, C. Thorn, T.E. Kocal and H.W. Ferguson. 1990. Pathogenesis of skin and liver neoplasms in white suckers from industrially polluted areas in Lake Ontario. Sci. Total. Environ. 94:105-123.

Heaton, S.N., R.J. Aulerich, S.J. Bursian, J.P. Giesy, J.A. Render, D.E. Tillitt, and T.J. Kubiak. 1993. Reproductive effects of feeding Saginaw Bay source fish to ranch mink. J. Great Lakes Res. 19, No. 4.

Henny, C.J. 1972. *An Analysis of the Population Dynamics of Selected Avian Species with Special Reference to Changes During the Modern Pesticide Era.* Wildlife Res. Rpt. 1, Bureau of Sport Fisheries and Wildlife, Washington, D.C.

Hodson, P.V. 1990. Indicators of ecosystem health at the species level and the example of selenium effects on fish. Environ. Monit. Assess. 15:241-254.

Hodson, P.V., B.R. Blunt, D.J. Spry and K. Austen. 1977. Evaluation of erythrocyte aminolevulinic acid dehydratase activity as a short-term indicator in fish of a harmful exposure to lead. J. Fish. Res. Board Can. 34:501-508.

Hodson, P.V., K. Ralph, L. Luxon, B. Gray and M. McWhirter. 1989. Mixed function oxidase (MFO) activity of Great Lakes fish. Page 236 In: Abstracts of 10th Annual Meeting of Soc. Environ. Toxicol. Chem., Toronto.

Hoffman, D.J., B.A. Rattner, L. Sileo, D. Docherty, and T.J. Kubiak. 1987. Embryotoxicity, teratogenicity, and aryl hydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. Environ. Res. 42:176-184.

Hoffman, D.J., G.J. Smith and B.A. Rattner. 1993. Biomarkers of contaminant exposure in common terns and black-crowned night herons in the Great Lakes. Environ. Toxicol. Chem. 12:1095-1103.

Humphrey, H.E.B. 1988. Chemical contaminants in the Great Lakes: the human health aspect. Pages 153-165 In: M.S. Evans (ed.), *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus.* J. Wiley and Sons, New York.

International Joint Commission. 1987. Revised Great Lakes Water Quality Agreement of 1978. Windsor, Ontario.

Jacobson, S., G. Fein, J. Jacobson, P. Schwartz, and J. Dowler. 1985. The effect of intrauterine PCB exposure on visual recognition memory. Child Develop. 56:853-860.

Jacobson, J. and S. Jacobson. 1988. New methodologies for assessing the effects of prenatal toxic exposure on cognitive functioning in humans. Pages 373-387 In: M.S. Evans (ed.) *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus.* J. Wiley and Sons, New York.

Kennedy, S.W. and G.A. Fox. 1990. Highly carboxylated porphyrins as a biomarker of polyhalogenated aromatic hydrocarbon exposure in wildlife: confirmation of their presence in Great Lakes herring gull chicks in the early 1970s and important methological details. Chemosphere 21:407-415.

Kerr, S.R. and R.A. Ryder. 1977. Niche theory and percid community structure. J. Fish. Res. Board Can. 34:1952-1958.

Kubiak, T.J., H.J. Harris, L.M. Smith, T.R. Schwartz, D.L. Stalling, J.A. Trick, L. Sileo, D. Docherty and T.C. Erdman. 1989. Microcontaminants and reproductive impairment of Forsters tern in Green Bay, L. Michigan - 1983. Arch. Environ. Contam. Toxicol. 18:706-727.

L'Arrivee and H. Blokpoel. 1988. Seasonal distribution and site fidelity in Great Lakes Caspian terns. Colonial Waterbirds 11:202-214.

Lilienfeld, A.M. and A.B. Lilienfeld. 1980. *Foundations of Epidemiology.* 2nd ed. Oxford University Press, New York.

Lindley, L.A., J.P. Giesy and T.E. Bryant. 1993. The caffeine breath test: a method to measure cytochrome P450-1A1 activity in birds. Page 113 In: Abstracts of the 36th Conference of the Internat. Assoc. Great Lakes Res., De Pere, Wisconsin.

Ludwig, J.P. 1979. Present status of the Caspian tern population of the Great Lakes. Michigan Academician 12:69-77.

Lugo, A.E. 1978. Stress in Ecosystems. Pages 62-101 In: J.H. Thorp and J.W. Gibbons (eds.) *Energy and Environmental Stress in Aquatic Systems*, Tech. Info. Cent., U.S. Dept. of Energy, Washington, D.C.

Luxon, P.L., P.V. Hodson and U. Borgmann. 1987. Hepatic aryl hydrocarbon hydroxylase activity of lake trout (*Salvelinus namaycush*) as an indicator of organic pollution. Environ. Toxicol. Chem. 5:649-657.

Mac, M.J. and C.C. Edsall. 1991. Environmental contaminants and the reproductive success of lake trout in the Great Lakes: An epidemiological approach. J. Toxicol. Environ. Health 33:375-394.

Mac, M.J., C.C. Edsall and J.C. Seelye. 1985. Survival of lake trout eggs and fry reared in water from the upper Great Lakes. J. Great Lakes Res. 11:520-529.

Mac, M.J. and M. Gilbertson (eds.). 1990. Proceedings of the Roundtable on Contaminant-Caused Reproductive Problems in Salmonids. Ecological Committee, Great Lakes Science Advisory Board, International Joint Commission, Windsor, Ontario. 44 pp.

Mac, M.J. and T.R. Schwartz. 1993. Investigations into the effects of PCB congeners on reproduction in lake trout from the Great Lakes. J. Great Lakes Res. 19, No. 4.

Marshall, R.R., R.A. Ryder, C.J. Edwards, and G.R. Spangler. 1987. Using the lake troutas an indicator of ecosystem health. Application of the dichotomous key. Tech. Rep. No. 49, Great Lakes Fishery Commission, Ann Arbor, Michigan.

McCarthy, J.F. 1990. Concluding remarks: implementation of a biomarker-based environmental monitoring program. Pages 429-439 In: J.F. McCarthy and L.R. Shugart (eds.), *Biomarkers of Environmental Contamination.* Lewis Publ., Boca Raton, Florida.

Maccubbin, A.E., S. Chidambaram and J.J. Black. 1988. Metabolites of aromatic hydrocarbons in the bile of brown bullheads, *Ictalurus nebulosus*. J. Great Lakes Res. 14:101-108.

Mora, M.A., H.J. Auman, J.P. Ludwig, J.P. Giesy, D.A. Verbrugge and M.E. Ludwig. 1993. Polychlorinated biphenyls and chlorinated insecticides in plasma of Caspian terns: relationships with age, productivity, and colony site tenacity in the Great Lakes. Arch. Environ. Contam. Toxicol. 24:320-331.

Mouw, D., K. Kalitis, M. Anver, J. Schwartz, A. Constan, R. Hartung, B. Cohen, and D. Ringler. 1975. Possible lead toxicity in urban vs. rural rats. Arch. Environ. Health 30:276-280.

Munawar, M. and I.F. Munawar. 1982. Phycological studies in Lakes Ontario, Erie, Huron and Superior. Can. J. Bot. 60:1837-1858.

Munkittrick, K.R. and D.G. Dixon. 1989a. Use of white sucker (*Catastomus commersoni*) populations to assess the health of aquatic ecosystems exposed to low-level contaminant stress. Can. J. Fish. Aquat. Sci. 46:1455-1462.

Munkittrick, K.R. and D.G. Dixon. 1989b. A holistic approach to ecosystem health assessment using fish popu-lation characteristics. Hydrobiologia 188/189:123-135.

Nalepa, T.F. and P.F. Landrum. 1988. Benthic invertebrates and contaminanat levels in the Great Lakes: effect, fate and role in cycling. Pages 77-102 In: M.S. Evans (ed.), *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus*. J. Wiley and Sons, Toronto.

NRC Canada. 1985. The role of biochemical indicators in the assessment of ecosystem health - their development and validation. Associate Committee on Scientific Criteria for Environmental Quality, National Research Council of Canada. NRCC, Ottawa.

NWF/CIELAP. 1991. A Prescription for Healthy Great Lakes. National Wildlife Federation, Ann Arbor, Michigan and Canadian Institute for Environmental Law and Policy, Toronto. 65 pp.

Poland, A. and J.C. Knutson. 1982. 2,3,7,8-Tetrachloro-dibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Ann. Rev. Pharmacol. Toxicol. 22:517-554.

Prater, B.L. and M. Anderson. 1977. A 96-hour sediment bioassay of Duluth and Superior Harbor basins using *Hexagenia limbata, Asellus communis, Daphnia magna,* and *Pimephalos promelas* as test organisms. Bull. Environ. Contam. Toxicol. 13:159-169.

Rattner, B.A., D.J. Hoffman, and C.M. Marn. 1989. Use of mixed-function oxidases to monitor contaminant exposure in wildlife. Environ. Toxicol. Chem. 8:1093-1102.

Reynoldson, T.B., D.W. Schloesser and B.A. Manny. 1989. Development of a benthic invertebrate objective for mesotrophic Great Lakes waters. J. Great Lakes Res. 15:669-686.

Reynoldson, T.B. and M.A. Zarull. 1989. The biological assessment of contaminated sediments - the Detroit River example. Hydrobiologia 188/189:463-476.

Rogan, W. and B. Gladen. 1985. Study of human lactation for effects of environmental contaminants: The North Carolina breast milk and formula project and some other ideas. Environ. Health Perspect. 60:215-221.

Rogan, W., B. Gladen, K. Hung, S. Koong, L. Shih, J. Taylor, Y. Wu, D. Yang, N. Ragan, and C. Hsu. 1988. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241:334-336.

Ryder, R.A. and C.J. Edwards. 1985. A conceptual approach for the application of biological indicators of ecosystem quality in the Great Lakes Basin. Report to the Great Lakes Science Advisory Board, International Joint Commission, Windsor, Ontario. 169 pp.

Scheuhammer, A.M. 1987. Erythrocyte aminolevulinic acid dehydratase in birds. II. The effects of lead exposure in vivo. Toxicology 45:165-175.

Scheuhammer, A.M. 1989. Monitoring wild bird popu-lations for lead exposure. J. Wildlife Manage. 53:759-765.

Schindler, D.W. 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44(Suppl. 1):6-25.

Schneider, S. and R. Campbell. (eds.). 1991. Cause-Effect Linkages II. Symposium abstracts. Workshop of the International Joint Commission, Traverse City, Michigan, September 27-28, 1991. Michigan Audubon Society, Ann Arbor.

Shugart, L.R. 1988. Quantitation of chemically induced damage to DNA of aquatic organisms by alkaline unwinding assay. Aquat. Toxicol. 13:43-52.

Sicko-Goad, L. and E.F. Stoermer. 1988. Effects of toxicants on phytoplankton with special reference to the Laurentian Great Lakes. Pages 19-51 In: M.S. Evans (ed.), *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus.* J. Wiley and Sons, Toronto.

Sprunt, A., W. Robertson, S. Postupalski, R. Hensel, C. Knoder and F. Ligas. 1973. Comparative productivity of six bald eagle populations. Trans. 38th N. Amer. Wildl. Nat. Resourc. Conf.: 96-108.

Sutter, G.L. II, 1990. Use of Biomarkers in Ecological Risk Assessment. Pages 419-426 In: J.F. McCarthy and L.R. Shugart (Eds), *Biomarkers of Environmental Contamination.* Lewis Publ., Boca Raton

Susser, M. 1986. Rules of inference in epidemiology. Reg. Toxicol. Pharmacol. 6:116-128.

Suter, G.L. II. 1990. Use of biomarkers in ecological risk assessment. Pages 419-426. In: J.F. McCarthy and L.R. Shugart (eds.), *Biomarkers of Environmental Contamination.* Lewis Publ., Boca Raton, Florida.

Temple, S.A. and J.A. Wiens. 1989. Bird populations and environmental changes: can birds be bio-indicators? Am. Birds 43:260-270.

Tretjak, Z., S. Beckmann, A. Tretjak, and C. Gunnerson. 1989. Occupational, environmental and public health in Semic: a case study of polychlorinated biphenyl (PCB) pollution. pp. 57-72. In: Post-Audits of Environmental Programs and Projects. Proc. Environ. Impact. Anal. Res. Council/ ASCE, New Orleans.

Virtual Elimination Task Force. 1991. Persistent toxic substances: Virtually eliminating inputs to the Great Lakes. Interim Report to the International Joint Commission, Windsor, Ontario. 42 pp.

Virtual Elimination Task Force. 1993a. A Strategy for Virtual Elimination of Persistent Toxic Substances. Volume 1. Report to the International Joint Commission, Windsor, Ontario. 72 pp.

Virtual Elimination Task Force. 1993b. A Strategy for Virtual Elimination of Persistent Toxic Substances. Volume 2. Appendices. Seven Reports to the Virtual Elimination Task Force. International Joint Commission, Windsor, Ontario. 112 pp.

Walker, M.K., P.M. Cook, A. Batterman, L.C. Hufnagle, R.E. Peterson. 1993. Toxicity of maternally-derived 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during lake trout early development. Page 110 In: Abstracts of 36th Conference of Internat. Assoc. Great Lakes Res., De Pere, Wisconsin.

Walker, M.K. and R.E. Peterson. 1991. Potencies of polychlorinated dibenzo-p-dioxin, dibenzofuran, and biphenyl congeners, relative to 2,3,7,8-tetrachloro-dibenzo-p-dioxin, for producing early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). Aquat. Tox. 21:219-238.

Walker, M.K., J.M. Spitsbergen, J.R. Olson, and R.F. Peterson. 1991. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity during early life stage development of lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. 48:875-883.

Warwick, W.F., 1988. Morphological deformities in Chironomidae (Diptera) larvae as biological indicators of toxic stress. Pages 281-320 In: M.S. Evans (ed.), *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus.* J. Wiley and Sons, Toronto.

Weseloh, D.V. 1984. The origins of banded herring gulls recovered in the Great Lakes region. J. Field Ornithol. 55:190-195.

Willford, W.A., R.A. Bergstedt, W.H. Berlin, N.R. Foster, R.J. Hasselberg, M.J. Mac, D.R.M. Passino, R.E. Reinert and D.V. Rottiers. 1981. Introduction and summary. Pages 1-7 In: *Chlorinated Hydrocarbons as a Factor in the Reproduction and Survival of Lake Trout (Salvelinus namaycush) in Lake Michigan.* Tech. Paper 105, U.S. Fish and Wildlife Service, Fort Collins, Colorado.

Wren, C.D. 1991. Cause-effect linkages between chemicals and populations of mink (*Mustela vison*) and otter (*Lutra canadensis*) in the Great Lakes Basin. J. Toxicol. Environ. Health 33:549-585.

Zile, M.H. 1992. Vitamin A homeostatis endangered by environmental pollutants. Proc. Soc. Exper. Biol. Med. 201:141-153.

DEFINITIONS AND GLOSSARY

Biochemical marker
A cellular or biochemical component, process, structure or function which responds to changes in the integrity or quality of the environment, particularly exposure to xenobiotic (man-made) chemicals.

Bioindicator
An organism and/or biological process whose change in numbers, structure or function points to changes in the integrity or quality of the environment. Bioindicators include sentinel or indicator species, biochemical markers, and biological effects.

Biological effect
A measurable change in some structure or function in an indicator organism which reflects changes in the quality of the environment. Examples are mortality, growth retardation, behavioural changes, malformation, embryo death, and tumors.

Biomonitoring
The regular collection of information on the biological structure and/or functioning of bioindicators to assess the quality of the environment.

Cause-effect linkage
The systematic application of epidemiological criteria to test whether hypothetical associations between observed changes in structure and/or function of bioindicators are attributable to a suspected causal agent(s).

Indicator or sentinel species
Organisms whose biological characteristics and ecological requirements enable quantitative measurements which reflect cultural stress and quality of the environment.

Scientific method
Principles and procedures for the systematic pursuit of knowledge involving the recognition and definition of a problem, the collection of data through observations and experiment, and the formulation and testing of hypotheses.

Weight of evidence
A summary assessment of cause-effect investigations on which policy decisions may be made.

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