EPA-600/4-80-

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BIOLOGICAL MONITORING OF TERRESTRIAL ECOSYSTEMS USING HONEY BEES AND EARTHWORMS

A Workshop Summary
and Panel Reports

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Contract No. 68-03-1526

Project Officer

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OFFICE OF RESEARCH AND DEVELOPMENT
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ACKNOWLEDGEMENTS

I wish to thank the workshop participants for their willingness to expend their energy and devote long hours to the production of two separate research projects to "prove" the utility of terrestrial biological monitoring.

I am indebted to J.B. States and E.M. Preston for their assistance in conducting the workshop.

I wish to acknowledge the members of the work groups:

Soils - J.B. States (Chairman), B.S. Ausmus, K. Cromack, Jr., C.D. Drewes, E.F. Neuhauser, and T.R. Seastedt.

Honey Bees - J.J. Bromenshenk (Chairman), D.M. Burgett, R.W. Ferenbaugh, Y.I. Lehner, L. Rogers and H. Shimanuki.

M. Ginevan, B.A. Kahn and E.M. Preston worked with both groups.

FOREWORD

PRESTON WILL WRITE, ATTACH IN CORVALLIS

ABSTRACT

One goal of EPA's Anticipatory Research Program is to identify and to characterize emerging environmental problems before they become serious. In view of this objective, EPA's Corvallis Environmental Research Laboratory will initiate a research program during fiscal year 1980 to develop biological monitoring methods for terrestrial ecosystems. Approximately half of the initial effort will be directed towards determining trends in the presence and amounts of toxic chemical residues in biota from a limited geographical area. If feasible, this endeavor could be expanded to a national monitoring network. The other half of the effort will focus on correlating tissue residue levels of these chemicals with physiological and ecological responses and will assess the utility of such responses for a "biological effects monitoring" program.

In March, 1980, a workshop was held in Corvallis, Oregon to discuss the implementation of a pilot program of biological monitoring using honey bees and soil invertebrates. Specialists in the fields of ecology, soil biology, apriculture, environmental problems, physiology, and biochemistry representing government, university, and private research affiliations convened to consider research and development needs, classes of chemicals and types of sites to be monitored, sampling designs, and methods of sample collection, handling, and analysis. A phased research program was designed for a short-term (one year) field, laboratory, and paper studies and for a long-term (four to five years) fully scaled field and laboratory endeavor. Specific projects were established for the first year effort, while guidance was provided at the level of detail necessary to establish goals, priorities, and direction of a long-term program. An important aspect of the program is an emphasis on linking toxic chemical levels to quantifiable physiological responses and to measurable effects on the structural and functional status of biological systems. The program will be reviewed frequently and the usefulness of biological monitoring organisms and methods reassessed.

This report was submitted in fulfillment of Contract No. 68-03-1526 by University of Montana under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from March 6, 1980 to April 4, 1980, and work was completed as of April 4, 1980.

CONTENTS

SECTION 1

INTRODUCTION

One of the goals of EPA's Anticipatory Research Program is to identify emerging environmental problems before they become serious or irreversible. In keeping with this objective, EPA's Corvallis Environmental Research Laboratory will initiate a research program during fiscal year 1980 to develop biological monitoring methods for terrestrial ecosystems. An important aspect of this program will be frequent review to determine the feasibility of specific biological monitors for assessing environmental quality.

Biological monitoring is a term which includes a wide range of activities. For the purpose of this report, biological monitoring is defined as the use of living material (populations, organisms, tissues) for the systematic determination of the presence and amounts of pollutants and/or the physiological and ecological effects of these substances as a function of space and time. From an ecological point of view, there are two types of biological monitoring: "active monitoring" which generally refers to the introduction of standard, managed organisms into ecosystems or communities to measure trends in the accumulation of hazardous chemicals and/or the responses of the organisms to these chemicals; and "passive monitoring" which usually relates to the use of indigenous organisms for similar purposes. The coal miner's canary in a cage is a familiar example of an active monitor. Plants such as pine trees, whose visible pathological symptoms often serve as the first indicators of air pollution, are examples of passive monitors.

EPA's decision to pursue a pilot program of biological monitoring appears to be a response to a rather sudden convergence of thinking and events. Each year, more and more hazardous substances are added to the environment. A list of the chemicals presently considered environmentally hazardous might contain thousands of compounds, and new compounds are constantly being introduced. Until recently, monitoring programs emphasized physical and chemical instrumentation to detect and precisely measure specific ambient contaminants. Refinements in analytical techniques have demonstrated that many substances are more widely distributed than previously reported. As the capability to measure smaller and smaller quantities of substances in the environment increased, the biological significance of these low concentrations became more and more uncertain. It also became apparent that humans and the environment are exposed not to single, independent substances, but to complex chemical mixtures. It follows that the combined effects of these substances can be examined only by looking at the responses of living components of biological systems. In addition, the emphasis of environmental research is, of necessity, shifting from observations of short-term, acute perturbations to long-term studies of the effects of low level, chronic exposures.

Thus, there is a growing awareness that standards and requirements for control technology must be based on information concerning the consequences of introducing various contaminants into ecosystems and the reasons why ecological perturbations should be kept within certain limits. In order to accomplish this, the capability must be developed to determine which ecosystem changes are "natural" and which are anthropogenically induced.

One indication of this awareness was the International Workshop on Monitoring Environmental Materials and Specimen Banking held in Berlin (West) in October, 1978. The conference was sponsored jointly by the Commission of the European Communities, Brussels, the Federal Ministry for Research and Technology, Bonn, the Federal Environmental Agency, Berlin, and the U.S. Environmental Protection Agency, Washington, D.C. A follow-up workshop was held in Germantown, U.S.A. in December of the same year. Their major conclusions were:

- Network systems should be established to monitor ecosystem exposure to substances which have, or may have, adverse effects.
- Specimen banks should be established for retrospective analysis of trends in exposures to previously unrecognized pollutants or pollutants for which analytical techniques may at present be inadequate.
- A number of plant and animal species appear to be suitable for biological monitoring and specimen banking.
- In the absence of effective monitoring programs, "the detection of serious environmental contamination from pollutants may occur only after critical damage has been done."

In 1979, EPA received two reports on the establishment of such a program: Goals of and Criteria for Design of a Biological Monitoring System by the Ecology Committee of the Science Advisory Board to EPA, and a Biological Monitoring Concept Paper presented by T.A. Murphy to the Blue Ribbon Select Committee on Monitoring. The two reports debated biological monitoring issues such as selection of species and sites, trophic levels, the general usefulness of any biological monitoring program, and monitoring of trends in hazardous pollutant residues versus biological effects.

Both reports concluded that biological monitoring procedures, on a selective basis, deserve considerably more attention by EPA. The ecology Committee of the Scientific Board commented that biological, physical and chemical monitoring are needed to examine changes in the structure and function of ecosystems because such biological monitoring is an "essential component of any monitoring program." The unique advantage of biological monitoring is that environmental regulations are designed to protect living organisms, not simply to limit chemical levels in the environment. Only biological monitoring can assess attainment or non-attainment of biological goals, including an effect such as toxicity or transport and fate processes such as accumulation and concentration.

As regards the monitoring objectives of EPA, these reports pointed out that biological assessments serve the same functions as chemical and physical determinations.

- Identification and definition of environmental problems - to reveal, detect, or anticipate environmental problems and to determine their nature, extent, magnitude and causes.
- Regulatory development - to provide the knowledge of causal relationships necessary for effective and reasonable regulations.
- Enforcement of regulations - to determine whether legal requirements are being met.
- Evaluation of regulations and programs - to evaluate the effectiveness of programs, regulations, or other abatement or control activity.

Although the reports were very informative, they did not provide specific guidelines for a biological monitoring program. There was some consensus that managed honey bees had immediate potential for network biological monitoring of toxic pollution exposures. Honey Bees were also identified as potentially good biological monitors at the International Workshop on Monitoring Environmental Materials and Specimen Banking. The two reports included little discussion of soils, but there was considerable discussion of the need for suitable soil monitors at the Berlin and Germantown Workshops. Earthworms were recommended for initial monitoring and banking, while microcosms and soils were suggested for later, long-term programs.

To address these issues more specifically, a workshop sponsored by EPA's Corvallis Environmental Research Laboratory was held in Corvallis, Oregon, March 18 through 20, 1980. Participants in the workshop are listed in Table 1. The purpose of the workshop was to design an Anticipatory Research Program of Terrestrial Biological Monitoring. The resultant pilot program is to assess the utility of specific biological tools (indicator species) for:

- Correlation of trends in toxic materials in the terrestrial environment and body burdens for use in a program of "bio-residue or exposure monitoring" in a limited geographical area. This forseeably could be expanded to a national monitoring network.
- Correlation of tissue residue levels of toxic materials with physiological and, if possible, ecological responses (changes in ecosystem structure or function) for eventual use in an "effects program."
- Acquisition of knowledge and understanding of the possible physiological mechanisms by which responses to a pollutant or pollutants are brought about and of the ecological significance of these responses.
- Determination of the feasibility, usefulness and accuracy of biological monitors for exposure monitoring, effects monitoring, and assessment of the condition or state of ecosystem health.

The pilot program is based upon a phased approach over four to five years. Phase I consists of short-term (approximately one year) field, laboratory and paper studies on the use of biological monitors (honey bees and earthworms) to determine trends in toxic pollutant residues in the environment and on physiological responses to these materials and some population responses. Expanding Phase I objectives, Phase II will begin to examine ecosystem effects at the level of functional and structural components. Phase III begins field tests and verifications of combined exposure and effects monitoring, using active and passive modes. Phase IV initiates the use of biological monitors in limited geographical areas for specific pollutant categories, thus providing background for possible national networks. Phases I and II were outlined by the workshop. Phases III and IV reflect long-term objectives, the specifics of which will depend on the outcome of Phases I and II.

The program will emphasize trends or links between toxic chemical exposure, physiological responses and ecosystem responses (changes in structure or function). Dose-response tests and correlations of body burdens with levels of pollutants in the environment and with observed effects are also important aspects of the pilot study.

The long-term program depends upon a reiterative process to review the potential usefulness of specific biological monitor species and methods. Workshops, research and development efforts, feasibility studies, and cooperative efforts through informal and formal contracts are important elements of Phase II, III, and IV. To ensure the continuity and direction of the long-term program, a core group of researchers, derived in part from the participants at this workshop, will oversee the program. Provisions were made for Program Advisory and Peer Review Groups.

This report serves as the proceedings of the workshop and presents discussions and conclusions reached in defining the phases of a pilot program of Terrestrial Biological Monitoring. Section 2 summarizes the overall workshop conclusions and recommendations. Section 3 presents a summary of general issues and long-term objectives, Sections 4 and 5 summarize discussions specific to each topic area: honey bees and soil organisms, respectively.

TABLE 1. TERRESTRIAL BIOLOGICAL MONITORING WORKSHOP PARTICIPANTS

Participant	Affiliation
Dr. Beverly S. Ausmus	Batelle Columbus Laboratories 505 King Avenue Columbus, Ohio 43201
Dr. Jerry J. Bromenshenk Workshop Chairman/ Coordinator	Environmental Studies Laboratory University of Montana Missoula, Montana 59812
Dr. D. Michael Burgett	Department of Entomology Oregon State University Corvallis, Oregon 97331
Dr. Kermit Cromack, Jr.	Forestry Science Research Oregon State University Corvallis, Oregon 97331
Dr. Charles D. Drewes	Zoology Department Iowa State University Ames, Iowa 50010
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TABLE 1. Concluded

Participant	Affiliation
Ms. Yolanda Lehner	North Central Bee Research 436 Russell Laboratory University of Wisconsin Madison, Wisconsin 53706
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SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

Recommended research projects for Phases I and II of a pilot biological monitoring program are listed in Table 2, which also presents specific experiments for each Phase I project. Details of Phase II projects are contingent upon the results of Phase I. Phases III and IV are covered in a more general manner because these stages depend to a high degree on the results of the first two phases and upon the level of funding.

It should be emphasized that the initial phases of the Terrestrial Biological Monitoring Program (TBMP) are highly exploratory. The concensus of the workshop participants was that honey bees and earthworms have a high potential as specific tools for this purpose because they are manageable and bioaccumulate many chemicals. Another selling point is that the information available on all aspects of their biology, as well as their interactions with pollutants, probably equals or surpasses that of any other invertebrates. Still, even for these organisms, experience with pollutant interactions has been limited mainly to acute exposures of a few toxic chemicals, and experience with long-term low level exposures to individual and chemical complex mixtures is almost non-existent.

Toxic heavy metals are emphasized in Phase I projects because small scale pilot demonstration projects should, of necessity, be relatively simple, easy to perform, informative, and cost effective. Other chemicals such as gaseous effluents, radioactive wastes, organic pesticides, and other organics including aliphatic, alicyclic, aromatic, and heterocyclic compounds received high priorities for examination in Phase II.

Any organisms recommended for biological monitoring after the early stages of the TBMP should be considered the best available at present, given the constraints of our knowledge and the resources available for feasibility studies. As our information base develops and our understanding of the use of biological monitors becomes more discriminating, other biological monitors may become more appropriate.

Specific recommendations and conclusions concerning the overall aspects of the TBMP are:

- A pilot program of biological monitoring of exposures and effects is needed and is technically feasible.

- Honey bees (*Apis mellifera*) and earthworms (*Eisenia foetida*) should be utilized in the initial phases of the program as biological monitors for two terrestrial media - air and soils, respectively.

- The integrity of the research packages is essential. Each package is aimed at providing information ranging from trends of toxic (includes mutagenicity, teratogenicity, carcinogenicity) pollutant residues in the environment, and resulting body burdens, all the way to the biological effects and their significance at the ecosystem level of organization.

- Research which provides integrated data from several levels of biological organization, in both structural and functional terms, represents an optimal approach.

- Initial validation of biological monitoring is contingent upon parallel experimentation with a functional ecosystem monitor and a biological monitor (e.g. earthworm/soil nutrient flux; honey bee/ pollination).

- Passive monitors are needed in addition to active monitors. Developing both types of monitors should provide effective tools for monitoring short-term perturbations (in the active mode) and long-term perturbations (in the passive mode), in the major ecosystems of concern.

TABLE 2. TERRESTRIAL BIOLOGICAL MONITORING RESEARCH PROJECTS

AIR MEDIA	SOILS MEDIA
PHASE I Field Tests - Honey Bees	PHASE I Lab Tests - Worms
o Bioaccumulation: Arsenic --Sampling Variance --Receptor Compartments --Correlations of Levels in Media and Bees --Other Heavy Metals	o Dose Response: Heavy Metals --Body Burden --Population Effects - Growth & Reproduction --Neural - Action Potential & Synaptic Transmission o Soil Nutrient Flux-Concept Paper --SNF as Ecological Monitor --Coupling Biological/Ecol. Monitors
Field & Lab Tests - Honey Bees o Dose Response: Arsenic --Reproduction/Mortality --Hoarding/Honey Production --Enzyme/Stress Indicators	PHASE II Lab Tests - Litter/Soil o Dose-Response: Heavy Metals --CO_2, DOC, PO_4-P or NH_3-N, NO_3-N --Sensitivity Analysis of SNF vs. Worm
PHASE II Field & Lab Tests-Honey Bees o Bioaccumulation: Organics/Inorganic o Refine Phase I Methods o Pollutant Transport/Cycling o Colony & Ecological Responses --Simulation Modelling --Pollination Syndrome --Effects of Pollutant Cycling --Food Chain Transfers --Stress Indicators	PHASE III Field and Lab Tests o Passive Monitors: Pollution Gradient --Soil/Litter Sampling - Elemental Analysis --Sampling of Worms - Elemental Analysis - Neurophysiological Tests
PHASE III Field & Lab Tests o Passive Monitors - Native Bees --Exposure Monitoring --Population Effects --Stress Indicators --Simulation Modelling/Megachilidae o Active Monitors --Field Validations of Techniques	o Active Monitors: Pollution Gradient --Soil/Litter Sampling - Elemental Analysis - Exposure Analysis - Worms - Effects Analysis - Worms - Functional Analysis - Soils (SNF With & Without Worms) --Other Species of Worms --Field Validations of Predictions
PHASE IV Network Monitoring o Active and Passive Monitors	PHASE IV Application to National Network

SECTION 3

PROGRAM OVERVIEW

The Terrestrial Biological Monitoring Workshop was to provide specific recommendations for a pilot monitoring program. Throughout the workshop, several issues or concerns of a general nature were raised, and they are summarized in this section.

3.1 CURRENT STATUS OF TERRESTRIAL BIOLOGICAL MONITORS

The first issue was the relatively undeveloped state of the art for using biological monitors in environmental assessments. Too often, biological monitors seem to have been selected in a serendipitous, unfathomable manner. Not only do we lack appropriate monitors for environmental problem solving but our ability to interpret the significance of observed responses usually is limited, even when considering monitors that have proven valuable. For example, the presence or absence of lichens can provide early recognition of pollutant exposure problems, but the significance of this response to ecosystem or human welfare is unclear.

Biological monitors must supply the scientific and technical information needed by EPA and other users; especially information ranging from trends in pollutant concentrations in the environmental media and resultant body burdens all way to biological effects and their significance at the ecosystem level. For the most part, these information needs are not met by current biological monitors. Based upon discussions of these issues, the workshop participants arrived at the following realizations:

- Better science needs to be applied to biological monitoring and environmental problem solving. Most present techniques are inadequate and have very limited usage. A rigorous, organized approach establishing clear statements of objectives and tests of suitable hypotheses will significantly improve our capability to develop useful, quantitative monitors.

- Biological monitoring should be based upon a new perspective, one that clearly identifies the biological level of organization from which inferences will be made and which focuses on links that can be made between levels of exposures to pollutants and effects upon the the structural and functional status of biological systems of interest.

- There is a need to produce quantitative information that can be used to judge the significance of observed responses and which can be entered effectively into the decision making process.

3.2 SELECTION AND USE OF BIOLOGICAL MONITORS

Workshop participants were concerned that the pilot program not merely establish more monitoring tools or ones of limited use for a few chemicals or sites. The goal of the TBMP is to significantly improve monitoring capability such as increased sensitivity, earlier warning of pollutant build-up or potential environmental problems, greater reliability, more cost-effectiveness, and better information, particularly on biological effects.

When selecting and using biological monitors, it is important to establish the objectives of monitoring: Is it to provide a biological indicator which displays a rapid, unambiquous response to contaminant levels below those which cause general environmental damage, or is it to identify quantifiable responses that can be related to the responses of the system and which correlate in an understandable manner to pollutant levels? Desirable properties of the first (often termed biological indicators) may not be the same as those of the latter (termed biological monitors). The two functions are not mutually exclusive, but they may not be perfectly correlated. For the purposes of this workshop, discussions were directed towards developing biological monitors, not just indicators.

Workshop participants were asked to examine organisms and tests in the context of exposure and effects monitoring used in passive and active modes. In addition, two types of monitors were considered for each anthropogenic input (pollutant): taxanomic and ecosystem monitors. A taxanomic monitor provides information about possible responses of a taxon (species, family, order) regardless of ecosystem context; for example, exposure monitoring of DDT in the eggs of birds. An ecosystem monitor provides information about structure or function of an ecosystem (a particular assemblage of interacting species) for each ecosystem type: for example, pine trees as monitors of coniferious forests. These categories are not mutually exclusive. In general, for taxanomic monitors, inferences usually can be made only as far as the population level, while for ecosystem monitors, inferences can be made to the ecosystem level.

The level of biological organization of interest affects the inferences that can be made. Generally, if we wish to have explanations for what is observed, we should examine the next lower level of biological organization and make observations at that level. On the other hand, if we are to make statements concerning the significance of our observations, we should look to the next higher level of biological organization. The problem is to identify information requirements and then to design studies and interpret the data appropriate for the level of organization about which the inferences will be made.

The following definitions were agreed upon at the workshop;

Exposure Monitors -- used to establish that exposures to anthropogenic pollutants have occurred and to establish trends over time and across space. Generally, this involves determination of tissue residues (body burden determinations) and incorporates three concepts: the types and amounts of pollutants that impinge upon the organism, the dose that the organism receives, and resultant body burden, assuming the material bioaccumulates.

Effects Monitors -- used to evaluate the effects of exposure on the structural and functional status of biological systems of interest.

Exposure monitors can be classed as accumulators, concentrators, and magnifiers. Bioaccumulation is the ability of an organism to concentrate a substance throughout its life so that the concentration continuously increases. Bioconcentration is the active concentration of a substance by an organism as a result of activities such as ingestion or absorption. Biomagnification is the step-by-step concentration of substances through successive trophic levels of food chains.

Exposure monitors can be further catergorized by:

- The time period during which the sample is exposed. Tree rings are useful for long-term, honey bees are useful for short-term (seasonal) studies. Only the queen lives for more than one year.

- Range of chemicals for which the monitor is useable; a specific chemical or a category such as inorganics, or many chemicals or categories such as inorganics, organics, radionuclides.

Selection criteria for exposure monitors include:

- Cheaper and/or more effective than physical/chemical methods.

- Accumulator and preferably a concentrator or magnifier to increase detection capability.

- Relatively insensitive to as many toxic substances as possible. An organism that dies when exposed to low levels of toxic materials has a very limited period of use.

- Widespread distribution, preferably nationwide.

- Important structural or functional component of ecosystems. If exposures can be linked to effects, so much the better.

Effects monitors can be categorized as:

- Threshold monitors or organisms which are extremely sensitive. If they do not respond, it, generally is assumed that the system is not responding.

- Quantitative monitors which display measurable responses that can be related to the response of the system.

- Known or suspected responses to pollutant.

- Occurs in the ecosystem of interest.
- Relevant to important ecosystem structures or functions.
- Appropriate level of biological organization.
- Process or resource of interest to human welfare - desirable.

An organism may not bioaccumulate a substance, yet may be very susceptible to harm such as poisoning. Conversely, many good bioaccumulators are relatively insensitive to injury from a variety of toxic substances. Thus, a good exposure monitor may not be a good effects monitor and vice versa, but if the two can be linked, that is a bonus.

The format presented in Figure 1 was used to guide workshop discussions for selection of candidate monitoring organisms and methods and as an aid in visualizing the relationships of the various concepts.

Figure 2 presents a flow chart depicting how information provided by a biological monitoring program can be incorporated into the decision making framework. Anthropogenic pollutants may cause perturbations to ecosystems which change measurable ecosystem properties or attributes. The responses of indicators are a function of the ecosystem properties, which can be described by process equations, i.e. $f(x,y)$'s and $g(x,y)$'s. All information flows ultimately lead to decision makers such as administrators, managers, scientific and technical staffs, and public interest groups. To insure that this occurs in an effective manner, the information obtained must answer the proper questions, including what needs to be known to facilitate environmentally sound decisions, how to design and how to apply the best monitoring techniques available so as to properly address specific statements of objectives, and how to put the information obtained into a useable format and in a timely manner so as to effectively influence decisions.

3.3 INTERFACES WITH OTHER PROGRAMS, AGENCIES, AND INDUSTRY

Each year, millions of dollars are spent on physical and chemical monitoring and environmental impact assessments. Numerous applied and basic research programs are attempting to develop the capability to predict the responses of ecosystems to various perturbations. The basic question is to provide questions about the consequences of introducing various quantities of pollutants into the environment.

For economy of personnel and financial resources, it is important to integrate EPA's terrestrial biological monitoring effort with other ongoing research. The information value of data gathered for the biological monitoring program will be greatly enhanced if the project were carried out in an area where extensive physical and chemical monitoring and/or ecological monitoring is being conducted. This would facilitate the understanding of interactions and relationships between the various aspects of the system and in doing so should increase the potential predictive value.

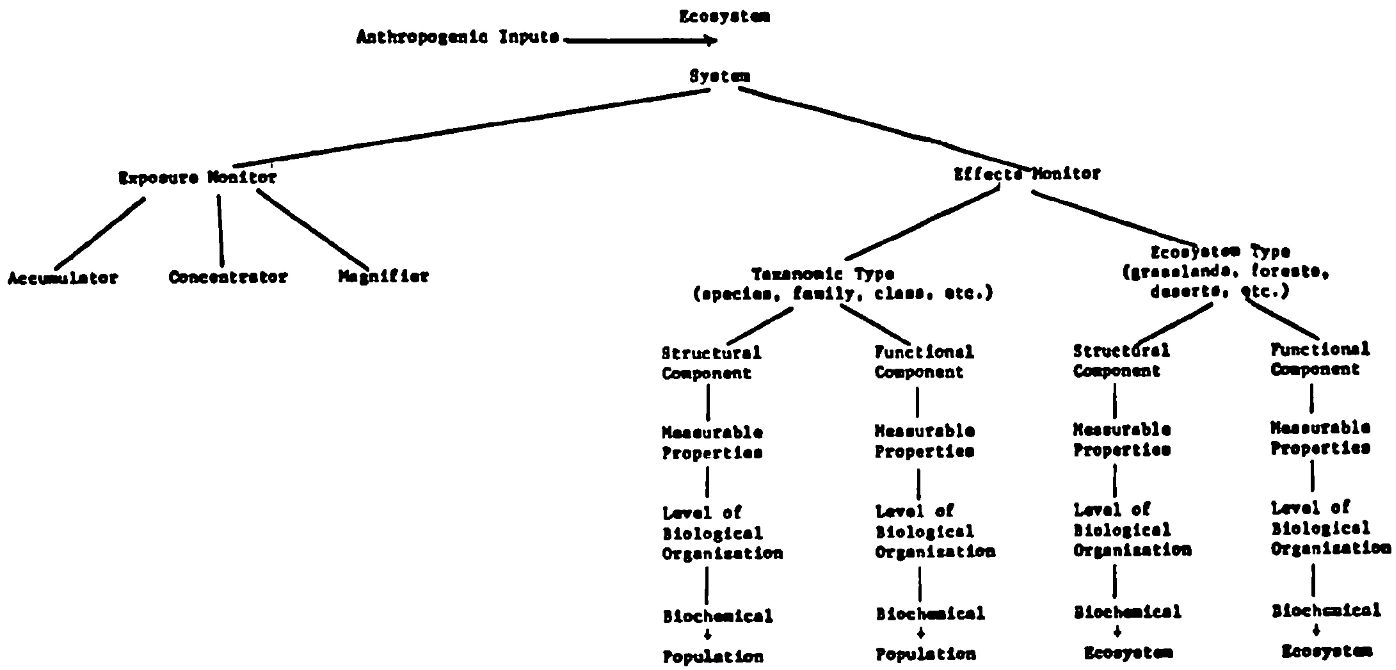

Figure 1. Selection of biological monitors.

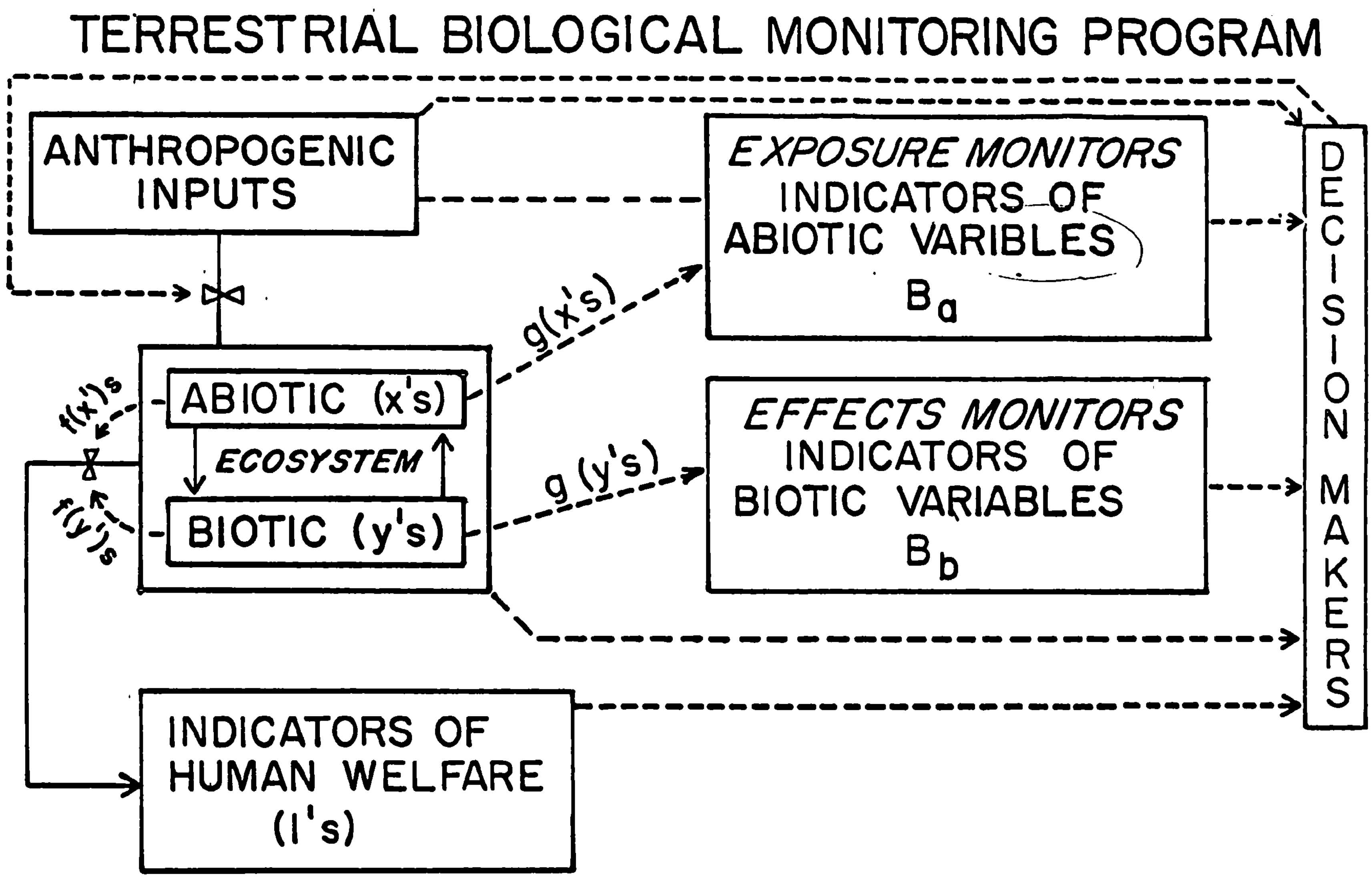

Figure 2. Biological monitoring in the context of the decision making framework. Solid lines represent energy flows, dotted lines information flows.

The TBMP is based upon a conceptual framework for international and national network monitoring of man's influence upon his environment (Luepke, 1979; Murphy, 1979; and Orians, 1979). The purpose of the TBMP is to demonstrate the utility of this concept through development of specific monitoring tools for use in such networks. One assumption made is that it is possible to select a minimum number of monitoring sites, "not an exclusive set," that provides a representative cross-section of major ecosystems of the United States (Botkin, 1978). Thus, site selection and characterization will become a major consideration at the point of applying these tools to network systems. Fortunately, that is precisely the issue being addressed by the Pilot Program for Long-Term Observation of Ecosystems (LTER) sponsored by the National Science Foundation.

EPA has several ongoing programs aimed at problem pollutants and their biological effects including acid rains (CERL), toxic substances (TOSCA), protocols for terrestrial ecological assessments (IERL-RTP), human effects monitoring (HEMP), pesticides (OPP), and solid wastes (OSW).

Department of Energy programs, especially as regards synthetic fuels and alternative energy technologies, have ongoing studies, several of which have established sites for long-term assessment of pre-operational and post-operational conditions. In cases such as these, DOE could profit by the addition of specific biological monitoring capabilities, while EPA would benefit by not having to locate sites or to characterize them.

There can be no doubt about the need for a capability to monitor accumulation and effects of anthropogenic pollutants. We now possess a conceptual framework for establishing network monitors. The initial strategy is to "prove" the utility of the concept by focusing on the most promising biological monitors for each of the two terrestrial media - honey bees in air and earthworms in soils. The objectives are to (1) correlate trends in toxic materials in the environment with body burden trends, (2) identify the biological responses to these trends, (3) understand the underlying biochemical and physiological responses, and (4) couple monitoring of important functional processes. If we can demonstrate that the technique works for bees and/or earthworms, it seems that a virtual flood of support for future development would appear from potential users.

3.4 PROGRAM MANAGEMENT

Proper program management is essential to maintain the focus on the purposes, goals, and objectives of the TBMP. Figure 4 outlines a proposed management structure. It is recommended that a core group of individuals from this workshop be retained in research and in a peer review or advisory capacity so as to preserve the integrity and continuity of the program and to provide direction and frequent review through each of the phases. The interagency advisory group will include potential users. This should guarantee that the information needs of the decision makers are addressed. It is the responsibility of the EPA project officer in cooperation with the policy advisory group to set and review program objectives. The project advisory group will meet semi-annually and will be composed of four to six principal investigators. Membership is expected to change as new projects are added and older ones are completed. The function of the peer review group is to critically assess products in view of stated objectives and to provide guidance in these matters.

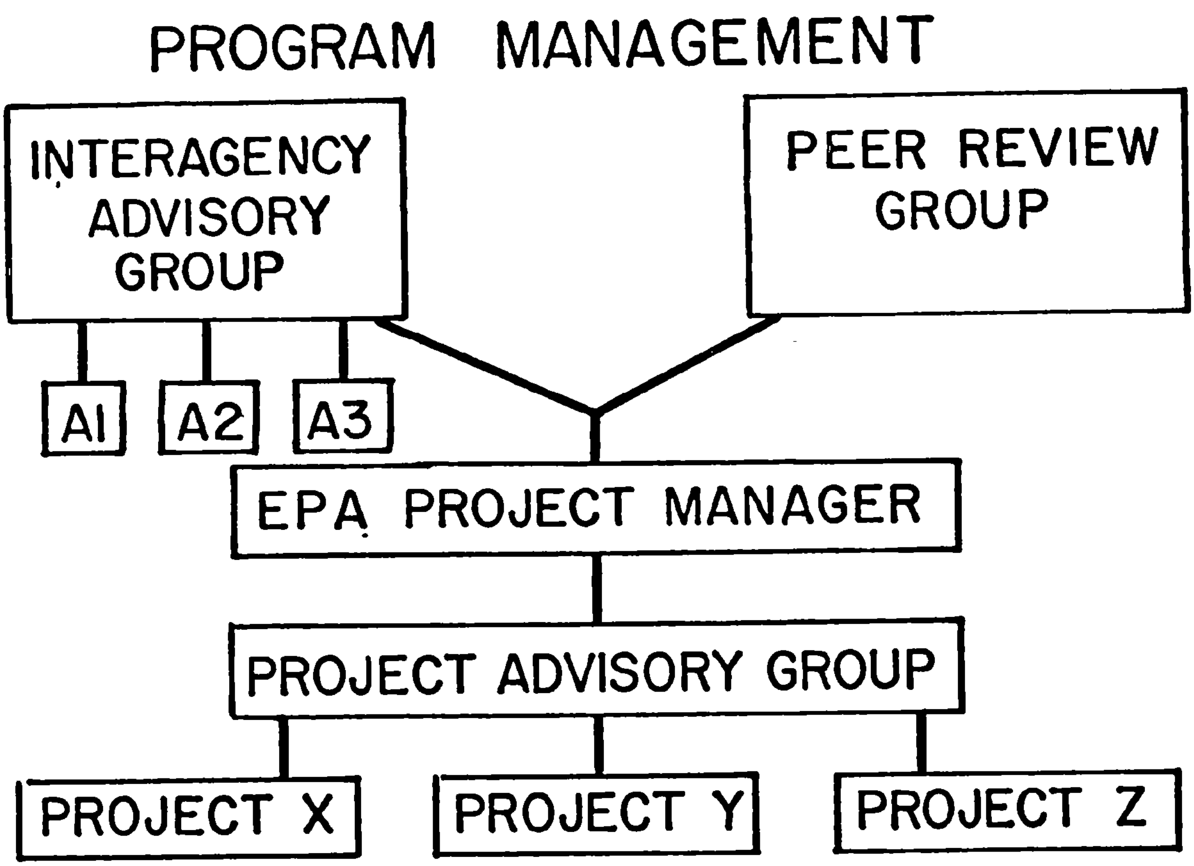

Figure 3. Management scheme for the Terrestrial Biological Monitoring Program.

SECTION 4

MONITORING THE AIR MEDIUM

Members of previous monitoring workshops and advisory groups proposed honey bees as good potential biological monitors, particularly for network monitoring of trends in toxic pollutant residues. Honey bees have been used for this purpose on a limited scale in the United States and in Europe. Because of these recommendations, a TBMP work group was formed, consisting of specialists in apiculture and environmental problems, the latter having experience with honey bees or native bees as biological monitors. After careful consideration, this group concurred that honey bees were ideal as part of an exploratory program to demonstrate the utility of the biological monitoring concept.

However, there are limitations to the use of honey bees. Honey bees are man-managed and as such can be deployed as active monitors. But the skill and knowledge of the person handling the experimental animal may greatly affect the colony. For example, poor management can lead to disease problems, low yields of honey and wax, or even death of the colony. Thus, during pilot phases of the monitoring program, a standardized agricultural methodology and experienced individuals are essential. In addition, many of the sampling techniques in a monitoring program might, at least for short periods, disrupt colony function.

4.1 RATIONALE FOR THE USE OF BEES

Despite these limitations,the consensus was that honey bees could greatly enhance a pilot biological monitoring program because:

- Bees (or other organisms) as biological monitors are preferable to chemical or physical instrumentation alone because they not only serve as bioaccumulators but also give some indication of physiological effects and provide information about the transfer of toxic materials through food chains and ecological effects.

- Since honey bees are man-managed, they can survive or thrive in nearly any biome. In addition, genetic variability can be reduced by using inbred lines and artificially inseminated queens. Thus, the honey bee possesses desirable characteristics for an active monitor.

- Honey bee colonies ~~Bees~~ magnify the level of many chemicals in their surroundings and increase the ability to detect pollutants.

- There is a potential for the transfer of harmful chemicals (toxins, mutagens, carcinogens) to humans via honey or pollen which should be addressed.

- The honey bee is economically important as a producer of honey and beeswax and for pollination service. A monitoring system using bees does not require transfer of the information (extrapolation or translations of the data) to be readily understandable in terms of consequences to mankind.

- The obvious link of honey bees to ecosystems is the pollination syndrome.

- Subtle effects of stressors, such as pollutants on bee populations, are often overlooked by scientists and beekeepers. A constant monitoring program should enable us to determine if changes in the productivity and efficacy of honey bee colonies occurs as a result of low level exposures to environmental contaminants.

4.2 PURPOSES OF USING BEES AS MONITORS

It became apparent early in the discussions that honey bees could provide two different services as biological monitors. Honey bees, as monitors, can provide an early warning of both pollutant accumulation and effects signaling possible harm to other organisms or to ecosystems. Especially important are interfaces with ecological processes. Another reason for using these organisms is to monitor hazards to the bees themselves and in turn to the beekeeping industry. As such, bees are a rather unusual biological monitor because they are not only sensitive bioindicators, but they themselves are a valuable resource.

4.3 MONITORING CONCERNS

A major difficulty in discussing the use of honey bees was that so much is known about them that it was not easy to prioritize the extensive list of possible parameters which could be utilized or the numerous methods or approaches. What the group needed to identify were simple, cheap and effective measurements useful in quantifying the biological effects of pollutants. Particularly important was the identification of parameters that control honey bee populations.

Dr. Shimanuki provided a particularly helpful tool — a simulation model of honey bee populations developed at the Bioenvironmental Bee Laboratory, Beltsville, Maryland. This model utilizes 22 parameters which can be readily quantified. Using the model and the collective experience of the work group participants, the discussion focused on interfacing the monitoring program with model development by providing data about pollutant effects on longevity, mortalities, reproductive cycles and foraging profiles. Testing the sensitivity of the various bee parameters that control the population model and providing the opportunity for basic studies to gain a better understanding of interactions are obvious benefits of working cooperatively on model development and monitoring. Once confidence is developed in the model, it can be used as a research tool to predict the impact of various chemical pollutants on populations of bees, impacts which could have consequences at the ecosystem level. Thus, the model became both a means of prioritizing the selection of parameters to be examined and a key element for later research phases of the TBMP.

A Biological Monitoring Research program would allow the addressing of pollutant effects on bee populations (commercial or managed honey bee demes), which may result in regional depressant effects. Long-term investigations of "sublethal," subtle, or chronic effects of exposures to anthropogenic materials may establish potential bionomic and economic depressant effects upon the bee industry and agriculture, such as increased costs of colony replacement, commercial pollinator shortages, predisposition of diseases, reduced honey and wax production, etc. (Figure 4).

An underlying assumption of the use of bees as monitors is that bees affected by a chemical will in turn reflect the fate of the colony. Examination of this assumption lead to two realizations:

- Evaluation of effects should be done on two levels -- the effect of the candidate chemical or chemicals on the individual bee and the effect on the colony.
- Except for the queen, the individual members of the hive do not reflect effects of the environment over long periods (greater than a year).

Thus, measures of the colony appear to be more appropriate than measures of individuals for monitoring impacts of chemicals over extended periods. The colony reflects more accurately direct cumulative effects.

In essence, when the honey bee is considered as a biological "tool," it should be remembered that the <u>colony is the organism</u>. The ultimate goal is to understand interactions of the pollutant with the colony as a whole and not just individual compartments (foragers, nurse bees, pupae, honey). For some purposes, such as exposure monitoring, one compartment may be of greater interest or utility than others, such as the use of honey versus bees, but, the colony generally is the basic unit of study.

Another monitoring concern discussed was that tests are needed which can easily and rapidly screen exposures and/or effects and be used in follow-ups on more specific concerns. Specific biochemical or physiological parameters could be selected to indicate the general health of bee parameters which remain relatively constant under a variety of climatic conditions. By establishing baseline values for these parameters and for the range of alterations in clean and polluted environments, we might be able to identify general physiological or biochemical stress indicators whose measurable alterations would indicate environmental hazards. Then, other specific tests could be used both on the bees and in the environment to delineate dangers. These stress indicators should remain relatively constant in bees even at low levels of honey production and/or pollination efficiency.

The use of honey bees as an environmental monitoring tool implies that some index of environmental conditions based on honey bee responses or activities would be developed and used in a predictive manner. Ecological indices generally require calibration before being used in this manner and this is not a trivial concern. Ebuchert *et al*, Batelle Pacific Northwest Laboratories were suggested as one of the major authoritative groups working on problems of this nature.

The discussions concerning honey bees as monitors focused on observable responses, their causes, their significance as indicative of ecosystem effects and the usefulness of all of these to decision makers. However, there was a concern expressed that we should not ignore possible threshold monitors that do provide an early warning of environmental perturbations. Honey bees may be excellent indicators in this sense, but the workshop discussions did not address this topic in any detail. That should not be construed to mean that this use of a biological monitor is not important.

Throughout these conversations, considerations of statistical design, adequate precision, statistical respectability, chemicals of interest, siting criteria, and interactions with other agencies and industry were addressed. Specifics of these discussions appear under the description of the short-term study plan in this section and in Sections 3 and 5.

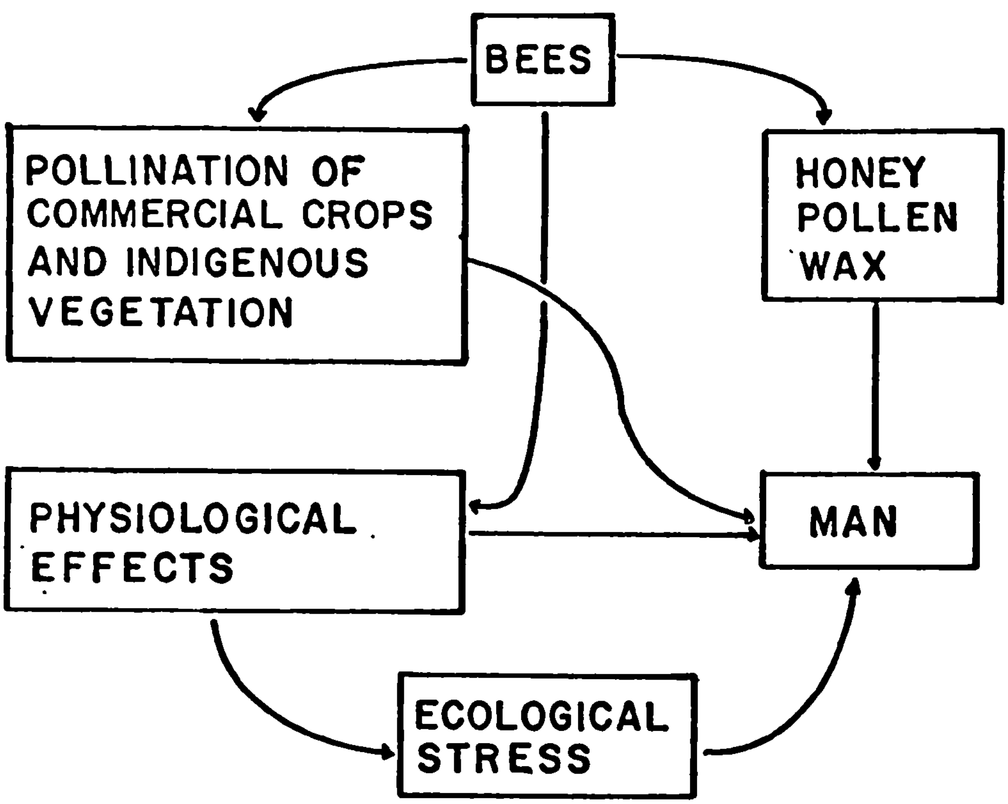

Figure 4. Conceptual model of key interactions between bees, the environment, and man.

4.4 PHASED RESEARCH PROGRAM

The bee work group produced a phased, four year research plan to perfect the use of honey bees and native bees as monitors of pollutant accumulations and effects. Major objectives of the program are to:

- Correlate trends in toxic substances in the environment with body burden trends (exposure monitoring).
- Identify biological responses to these trends (effects monitoring).
- Ascertain the physiological and biochemical response mechanisms involved.
- Provide integrated information concerning responses to pollutants at several levels of biological organization from the biochemical to the ecosystem.
- Develop models to examine and to predict the impact of various contaminants on populations of bees, on structural and functional status of ecosystems, and on valuable resources.

The work group believes that these objectives are attainable based on their collective expertise in the use of insects in exposure and effects monitoring, modelling of bee populations, bee life cycles, and population dynamics, bee diseases, bee physiology, biochemistry, and toxicology, insect ecology, and apiculture.

In order to achieve this goal in a timely manner, the initial effort should focus on an approach that has a high probability of success, yet which will address as many of the major objectives as possible.

Monitoring accumulation and effects of inorganic metallo-compounds in honey bees located near a known emission source seems to be a good starting point for demonstrating the utility of the biological monitoring concept. In addition, it provides an ideal opportunity to establish a small scale monitoring program in the field.

Arsenic, cadmium, lead, and other metals build-up in bees, pollen, and honey (Bromenshenk, 1980). There are reports dating to the early 1900's of severe bee kills near smelters and other arsenic sources. Although toxic effects of heavy metals are well known, little information exists about sublethal effects on colony health. Arsenic may be a continuing problem to the beekeeping industry. Members of the bee work group recently examined suspected chronic and/or acute arsenic poisoning of bees near industrial sources or areas in which arsenical defoliants had been applied.

The recognition of arsenic as a major environmental pollutant is growing. Bencko, 1977, reviewed the history and present status of knowledge concerning carcinogenic, teratogenic, and mutagenic effects of arsenic in humans. Thus, a demonstration project focused on arsenic could provide valuable basic information concerning a serious environmental threat.

Hopefully, the first-year monitoring program will successfully define exposure levels of individual honey bees (or groups of foragers) and of the colony as a whole, give a more complete picture of arsenic's mode of action, and show precisely what effect various levels of arsenic have on bees and colonies; i.e. critical dose-response relationships such as affects on mortality, longevity, and reproduction. The long-term project will address effects of arsenic and other pollutants at the population and ecosystem levels and eventually will examine regional impacts via network monitoring, at least on a limited scale. Beginning with Phase II, other chemicals such as organic compounds, radionuclides, and/or gases will be included. By Phase III, native bees will be incorporated as passive monitors to compliment honey bees as active monitors.

4.5 PHASE I - FIELD STUDIES

In electing to pursue a field trial, the work group expressed the belief that it already was feasible to utilize honey bees as exposure monitors, at least for certain chemicals. Earlier workshops had identified honey bees as promising tools for network toxic residue monitoring. However, honey bees have only been used for this purpose in a limited number of cases and usually for relatively short periods.

4.6 PHASE I - EXPOSURE MONITORING

The purpose of Phase I (Table 3-page 28) exposure monitoring tests is not just to show that bees accumulate arsenic or other heavy metals. The major objectives are to evaluate questions of deployment, sampling inter- and intra-colony variability, smallest representative samples, minimum levels of pollutant detectability, points most appropriate to monitor (foragers, nurse bees, pupae, pollen, or other), and correlations of pollutant levels in bee colonies with levels in the ambient environment.

Chemicals of Interest

Arsenic was selected as the primary chemical of interest for the initial effort because:

- It is magnified by bees.
- It is toxic but with a range of accumulation sufficient to allow monitoring of fairly long-term, sublethal effects.
- It is distributed throughout the hive compartments — so that accumulation and effects can be identified, studied, and quantified at several points for a better understanding of the interactions with bees and the colony as a whole.
- There is the possibility of food chain transfer of arsenic via pollen and honey.

Other heavy metals are of interest and should be included in analyses for the same samples, if cost restraints permit. Organic chemicals, particularly microencapsulated insecticides including Penncap-M®, Penncap-E®, and encapsulated Diazinon® and ~~the carbaryl~~ Sevin® received a high priority for inclusion to the program because these chemicals severely damage bees. Although ongoing programs are examining these chemicals in bees, the identification of encapsulated versus emulsified formulations of parathion and diazinon is very difficult. Insecticide usage usually results in hot spots or localized problems which may occur as a single event (e.g. grasshopper control which usually depends on one application during the growing season) or as a series of events (e.g. aphid control which may rely on weekly spraying). However, it should be feasible to produce a paper showing the role of bees as effects monitors of lethal and sublethal levels of insecticides based on a literature survey of current papers.

Other organic compounds such as PCBs, PAHs, or airborne hydrocarbons associated with the conversion of coal to synthetic fuels are of great interest. Many toxic, mutagenic, or carcinogenic organic chemicals of great concern to human health have never been examined in bees either from the viewpoint of possible bioaccumulation or adverse effects. We need to demonstrate the efficiency of bees in monitoring these other types of chemicals and sources. The work group felt that it was essential to design a bee monitoring program for a wide spectrum of pollutants and sources, not just a few chemicals or particular sites.

Criteria for Site Selection

The choice of the site for the short-term study should take into account the following:

- Known source of arsenic and other inorganic toxins. Sixteen copper smelters, a couple of chemical plants, and some geothermal sources are possible candidate sites.

- Point source clearly definable and surrounded by a relatively "pristine" environment so that sampling can be done along an exposure gradient within an area of similar climate and vegetation.

- Site well characterized in terms of available biotic and abiotic data. Many of these types of point sources have been studied over long periods of time and may have ongoing research and physical monitoring programs, especially of air quality.

Site selection becomes even more important in designing long-term exposure or effects monitoring programs. Sites must have integrity (protected from the public) and must be accessible. For long-term studies, permanent sites are essential. Availability of support data affects not only expenditures, but also the types of questions that can be addressed. Given the costs of characterizing complex systems, ties with other programs could be invaluable. As an example, there is an ongoing ecological monitoring program throughout the Northwest at several of the research natural areas. Other sites have been established at existing or planned fossil fuel and other technological development areas. The honey bee monitoring and possibly the soils (earthworm) monitoring could greatly enhance these programs at minimal expense to EPA.

Use of sites in agricultural areas versus less disturbed areas was debated at length. One participant commented that a demonstration of high pesticide levels within honey bee colonies located next to areas frequently sprayed was not going to impress anyone. But pesticides are hazardous to beekeeping, to human health, to insect pollinated crops. For these reasons, pesticides and agricultural areas deserve attention, especially as regards long-term effects of low level exposures. Also, agricultural systems may be the most relevant ecosystem for monitoring by honey bees because of the close interactions of bees and commercial crops. Unfortunately, pesticide impacts are complex issues that pose difficult problems in planning field exposure tests.

Field Design of Exposure Experiment

The recommended field design for the short-term exposure study (Table 3) calls for a 15 week study using honey bee colonies at a minimum of three sites arranged along an exposure gradient relative to an arsenic point source, such as one of the nation's sixteen copper smelters. At each site, colonies will be established in new equipment using package bees from a "clean" area. Sister queens, in bred-lines, and artificial insemination could minimize genetic variability. Samples of bees, pollen, and honey will be taken at three-week intervals - the development period from egg to adult. Five colonies per site were recommended, but the final number should be based upon literature reports and/or preliminary sampling of existing colonies in the study area to determine the number of observations (colonies sampled) needed to obtain a mean arsenic value with a desired confidence interval (prescribed base).

Phase I exposure studies rely upon the analysis of biological samples for arsenic content. These may reflect the fact that foraging bees visit different sites and as such are subjected to widely varying levels of arsenic contamination. Further, this contamination may be non-randomnly distributed through time, space, and within the colony.

The total number of bees or amount of pollen or honey which can be removed without seriously disrupting the colony is limited. The statistical problem is to apportion and to minimize both inter- and intra-colony variability and to determine the number of individuals (bees, pollen pellets, etc.) required to form a sample unit which adequately reflects the level of arsenic in the compartments of the colony. It is expected that as the number of bees in a sample increases, the average level per bee will converge to the average of the colony.

Similar statistical questions apply to the number of colonies sampled per site and the number of replications per compartment (e.g. bees, pollen, honey). Again, the problem is to determine what number (N) is needed to produce an acceptable standard error (S.E.). Published data (Bromenshenk, 1980) suggests that as regards the colonies at a site, the S.E. is a constant function of the mean arsenic content and that hive to hive variability may be a major source of error.

Specific statistical suggestions discussed by the work group included logarithmic transformation of data, the approach described by Pielou, 1969 for selection of quadrate size in environmental sampling, and the application of analysis of covariance and anova models to the data.

The work group recommended that at least four compartments of each colony be sampled for each site and date. These include foraging bees, nurse bees, pupae with pigmented eye capsules, and pollen. Other compartments of interest include larvae, eggs, and both capped and uncapped honey. Honey is of particular interest because of potential food chain transfer of arsenic to humans. Workshop participants reported that there was no reliable method to accurately determining arsenic concentrations in honey. If this analytical problem can be resolved, honey should be sampled. It might be advisable to collect and store honey for analysis at a later date.

Pupae and nurse bees will be collected from the brood frames within each hive. Pollen and foragers returning from the field will be obtained from traps attached to hive entrances. Top entrances are recommended for obtaining samples free of debris. Combination traps for both bees and pollen could be left in place on the hive with a baffle to direct bees into or past the trap.

At least one colony per site will be intensively sampled at a late enough date in the season that bioaccumulation should have occurred. A recommended procedure is to take a large sample, 1200 bees for example, at the entrance to a hive. In the laboratory, using tables of random numbers, twelve samples of 100 bees could be drawn for analysis. Following a similar procedure, the bees could be recombined (jackknifed) into samples of 200, 300, 400, or 600 for analysis. The purpose of the exercise is to determine for each compartment of the colony the smallest sample necessary for representativeness, accuracy, and precision.

At each site, air, water, and vegetation will be sampled. Soil samples could be included, but the work group did not think that soils would contribute much to the transfer of arsenic from the environment into bee colonies, except possibly by re-suspension under windy conditions.

Ambient arsenic is assumed to be an important entry route into bee systems. Air samples will be obtained using samplers such as Hi-Vols. The work group suggested utilizing at least four per site arrayed in a square, one sampler at each corner of the square, the hives at the center. Each air sampler should be located within the forage area of the bees, probably not more than one kilometer from the hives. Specifics of the air sampling program such as deployment, number of samplers, frequency of sampling, period of exposure, and even the type of sampler utilized, are left to the investigator performing the experiment since site factors will have to be incorporated in the design of an appropriate sampling scheme.

Water and vegetation are assumed to be other major routes of entry. Water and floral resources utilized by bees are easy to identify by bee flight activity. Water and vegetation will be sampled at least every three weeks. Vegetation sampling will focus on the flowers visited by the bees, especially at points near the colonies and each of the air samplers.

For any environmental sampling, the program must be fully designed before the first sample is obtained from populations in the field in order to obtain a truly representative sample. Individual, population, pollutant, and site characteristics all interact in a dynamic framework, and each produces intrinsic variability. Thus, the value of any sample is limited to the extent to which it is truly representative of the set from which it is obtained. In addition, procedural sources of error introduced during collection, handling, storage, and treatment of samples before analysis must be adequately addressed to assure valid results. This quality assurance cannot be over-emphasized but the work group could only underscore its importance and identify major concerns given the time constraints of a three day meeting to address a four to five year program.

The research project proposed for Phase I is intended as a guideline for the minimum acceptable sampling program. Lower limits were set based on projected cost constraints. Fewer sites, colonies collections, or compartments could negate the experiment's value. This project must be reasonably funded to assure quality. More sites are strongly recommended and options such as more colonies at three or four sites or three colonies at more sites should be carefully evaluated. Also, Hi-Vols may not be the best physical monitors for arsenic. It was suggested that copper smelters may release appreciable amounts of arsine. If this is correct, some type of bubbler sampler may be needed to assess air quality.

The major objectives of Phase I exposure monitoring are to determine:

- How to best deploy and calibrate honey bees as biological monitors.
- Which compartments within the colony should be monitored.
- How physical measurements of environmental quality compare to biological measurements.

If this can be accomplished, procedures can be refined in Phase II, tested for other heavy metals, and hopefully extended to other chemicals of interest such as organics.

4.7 PHASE I - EFFECTS MONITORING

Lethal dose values of arsenic are well-established by field and laboratory studies, but it should be remembered that little is known or understood about the effects of these materials upon bee populations. This is especially true for dosages less than "acute." Therefore, any monitoring program which attempts to define effects must have as its antecedent a laboratory studies program which attempts to define under carefully controlled conditions the causal relationships between pollutant and colony health. If individual bees or colony health are to be utilized as a measure of effects of a given pollutant in a given environment, it will be very important to measure baseline health parameters in the same or an identical environment free of the pollutant.

Laboratory Studies of Population Effects

Based on ease of manipulation, ability to control conditions, reduction of sources of variance, and cost, a laboratory approach is recommended for Phase I effects studies (Table 3). Follow-up field studies and field validations are strongly recommended for Phase II.

TABLE 3. RECOMMENDED BEE RESEARCH PROJECTS

PHASE I

EXPOSURE MONITORING		EFFECTS MONITORING	
Field Studies of Arsenic Exposures and Resultant Body Burdens		Field Studies of Effects of Arsenic	
o Sampling Variance	(1)	o Mortalities (Dead Bee Traps)/ Population Size (Estimates)	(1)
--Intra- and intercolony			
--Sites		o Numbers of Eggs/Larvae/Pupae	(2)
--Dates		o Weight of Colony (Honey Yield)	(2)
o Receptor Compartments of Colony	(1)		
--Foragers		Laboratory Studies of Physiological or Biochemical Responses to Arsenic (Graded Dosages)	
--Nurse Bees			
--Pupae			
--Pollen			
--Smallest Representative Sample		o Pyruvate Metabolism	(1)
o Levels in Ambient Environment Versus Bee Systems	(1)	o Activity of Detoxifying Enzymes	(2)
--Air, Water, Vegetation of Colony		Laboratory Studies of Population Responses to Arsenic (Graded Dosages)	
o Bioaccumulation of Heavy Metals	(2)		
--Cadmium, Lead, Copper			
		o Mortalities/Longevity	(1)
		o Numbers of Eggs/Larvae/Pupae	(1)
		o Hoarding Behavior	(2)
		o Longevity of Field Exposed Bees	(2½)

Ranking of Priorities: (1) Highest, (3) Lowest.

The laboratory study focuses on critical dose-response relationships: mortality, longevity, reproductive cycles, and food storage. No work on arsenic clearly shows what effects chronic levels have on the bee. The most fundamental research needs to be done on mortality and longevity of adult bees at graded doses. Colony effects also need to be established because longevity of adults by itself may not be an adequate indicator of damage. Either the queen or the younger stages may be more susceptible to arsenic levels than the workers. Thus, reproductive processes including numbers of eggs, larvae, and pupae will be measured. Harm to the queen could be reflected by depressed fecundity, fewer viable eggs, or a greater proportion of drones to workers (if sperm was affected). Lethal factors affecting any of the development stages are expected to alter the number of individuals reaching the next development stage (e.g. egg - larvae, larvae - pupae, pupae - adult).

Initial screening will be done in a greenhouse using a linear or logarithmic progression of ten doses of arsenic mixed with the food source (pollen or honey). The doses will range from zero to nearly lethal. Colonies will probably be 4-frame "nucs" made up of one pound of bees. Mortality, longevity, numbers of, eggs, larvae, and pupae, and hoarding activity will be determined bi-weekly for eight to twelve weeks. Hoarding is a laboratory measure of syrup storage which indicates the colony's inclination towards gathering and storing honey.

It is the colony as a whole that is responsible for honey production and pollination. Reproductive failure or mortality results in smaller field forces available for gathering nectar and pollen and for pollination service. In terms of energy balance, these effects would require the colony to allocate more energy to producing workers and less to provisioning the hive.

The proposed Phase I laboratory study did not directly address resultant body burdens. This might be a worthwhile addition. Currently, a master's thesis is scheduled for completion at the University of Montana, Spring, 1980 that specifically examines graded doses of arsenic, LD_{50}'s, and resultant body burdens. In addition, an intensive study of uptake, partitioning, and cycling of arsenic and/or radioisotope tracers is projected as part of Phase II or III investigations.

Field Studies of Population Effects

Although Phase I effects monitoring emphasizes a laboratory study, the work group thought it desirable to carry out a few, relatively inexpensive, easy to perform tests in the field which would couple the field exposure monitoring project to the laboratory effort. Colony responses to arsenic in a field setting may differ radically from greenhouse responses. In the field, arsenic has multiple routes of entry and variable levels of exposure across time and space. Also, many more abiotic and biotic stressors impinge upon the colonies under field conditions.

Phase I field tests consist of population estimates, mortalities as reflected by numbers of bees in dead bee traps, and numbers of eggs, larvae, and pupae. In the field, the numbers of eggs, larvae, and pupae can be determined quickly by photographing the brood frames so that counts can be made at a later, more convenient time.

A lower priority project for Phase I which is recommended if funding constraints permit, is a longevity test in which frames of brood exposed at the field sites will be brought into the laboratory, ~~hatched~~ under controlled conditions (growth chambers), and longevity from time of ~~hatch~~ measured.

The work group also strongly recommended weighing the colonies on each sample date to obtain an estimate of honey storage. By itself, this data may be of little value, but if a long-term exposure monitoring program were initiated at these sites, than the first year's data could be an important point of reference. Portable scales are readily available for this purpose. It is even possible to set each hive on a scale as a base, although this increases costs substantially.

Laboratory Studies of Physiological/Biochemical Responses

As mentioned before, we need general biochemical or physiological stress indicators whose measurable parameters could be altered to monitor situations in which pollutants are either unknown or extremely mixed. Candidate "health" parameters include enzyme levels (Ache, transaminases, aconitose), immunoglobin patterns as an indicator of disease resistance, and measures of reproduction such as egg hatch or sperm production. Unfortunately, not much work has been done in this area with respect to bees and pollutants.

A first step is to identify the indicators which are most sensitive. For this, we can draw upon the mountain of data available from work on vertebrates. Few colonies of bees will be needed, and many of the assays involved are already simplified and available in kit form as a result of vertebrate research. As such, the screening for sensitive biochemical and physiological indicators should be relatively straightforward and relatively inexpensive,

Phase I activities include a concept paper synthesizing current knowledge or mode of action and on sensitive biochemical and physiological indicators for heavy metals.

Two lines of investigation seem to be particularly relevant and warrent immediate laboratory study -- effects on pyruvate metabolism and on microsomal enzyme detoxification systems.

We know arsenic is a stomach poison and that it uncouples oxidative-phosphorylation - a vital process of intracellular respiration, which occurs within the mitochondria of the cell. Rat studies demonstrate that arsenic affects pyruvate-dehydrogenase functioning, not by inhibiting the enzyme, but by working on the substrate. This can be examined by measuring ratios of effects on pyruvate metabolism. What is needed is transfer of rat liver and intestinal methodology to bees, which should be fairly easily accomplished.

An interesting and possibly more useful test is to assay the activity of detoxifying enzymes associated with microsomes. Again, rat studies indicate that arsenic at low levels confers some tolerance to pesticides, presumably by stimulating the activity of these enzymes. This suggests the possibility that low levels of arsenic may affect the ability of bees to tolerate other stresses. The techniques appear to be available to examine this phenonenon with respect to arsenic and bees and may be transferable to other compounds. If successful, this might provide a very useful, general stress measure.

We already know that many of the vertebrate assays can be modified for use with bees. Conversely, alterations in physiological or biochemical parameters indicative of the general health of the bee may eventually be of great utility not only for effects monitoring using bees but also for yielding insights into general mechanisms of biochemical and cellular level action of pollutants in vertebrates.

PHASE I - COST ESTIMATES

The bee work group did not attempt to produce detailed cost estimates. Field exposure trials may involve 1,000 to 5,000 or more analyses of samples for a single element - roughly estimated at $14.50 to $16.50 per sample for arsenic alone; $10.00 to $16.00 for each additional element. Another major outlay would be for 16 or more Hi-Vol samplers at $375 to $650 each. The 16 to 20 honey bee colonies, including bees and equipment cost $95.00 to $190.00 each, depending on whether honey supers are included. Electric power, stands for Hi-Vols, possible site rental, travel, and per diem are major field expenses for approximately 20 weeks. During the field period, two technical staff members would be needed, one to care for and sample bees, the other to maintain the air samples on a daily basis.

Given the cost outlays to set up the experiment and to obtain samples, the work group recommends analysis for more than one heavy metal. Often the same preparation and digestion procedures can be utilized for several metals.

The laboratory studies performed at a properly equipped institution are relatively inexpensive. Both of the Phase I laboratory projects could be performed by graduate students under the close supervision of a principal investigator. Rough cost estimates for measuring the population responses to graded doses of arsenic were from $7,500 to $10,000. The enzyme study would cost approximately the same in terms of staff and general equipment. The cost of a centrifuge was projected as a major equipment outlay of from $5,000 to $10,000.

PHASE II - FIELD AND LABORATORY STUDIES

Phase I activities are designed to mesh together, combining exposures, bioaccumulation, physiological/biochemical modes of action, and population effects.

Phase II (Table 4) addresses the significance of these biological responses in terms of honey bee systems and ecosystems. Phase I financial constraints precluded experimentation with many chemicals or with a functional monitor (pollination) in parallel with the biomonitor (honey bee). Nor was it possible to pair an active biomonitor (honey bee) with a passive biomonitor (native bee).

PHASE II - EXPOSURE MONITORING

During Phase II, chemicals other than inorganics will be included. If bees are to be useful exposure monitoring tools, we must be able to use them for a broad spectrum of chemicals. There are ongoing studies of bioaccumulation of radionuclides in bees and honey at Los Alamos Scientific Laboratories, Batelle Pacific Northwest Laboratories, and Oak Ridge National Laboratories. The bee work group recommends cooperative effects with these laboratories.

TABLE 4. RECOMMENDED BEE RESEARCH PROJECTS

PHASE II

EXPOSURE MONITORING	EFFECTS MONITORING
Field and Laboratory Studies	Field and Laboratory Studies of Effects on Honey Bee Systems
o Chemicals other than Metals (1) --Pesticides --Aliphatic, Alicyclic, Aromatic Heterocyclic Compounds --Radionuclides --Gases	o Whole Colony --Honey Bee Simulation/Model Adaptation and Testing (1) --Impacts of Cycling of Hazardous Chemicals (1) --Food Chain Transfers to Man of Toxic or Mutagenic Materials (1)
o Refinements of Phase I Techniques (1) --Temporal and Spatial --Sampling Variability --Ratios of Change	o Compartments/Individuals (2)
o Mass Transport, Cycling of Pollutants from Environment to Bees (1) --Radiotracers --Electric Charges on Bees and Pollutants	Field and Laboratory Studies of Effects on Ecological Systems o Pollination Syndrome (1) o Honey Bee Simulation Model/ Adaptation and Testing (1) o Impacts of Cycling of Hazardous Chemicals (1) o Biochemical/Physiological Responses to Various Classes of Chemicals. (1)

Ranking of Priorities: (1) Highest, (3) Lowest.

Organics are another high priority class of substances to add to Phase II. Whether pesticides (insecticides or herbicides) or organics such as those released by new energy conversion technologies should be utilized was unresolved. There are many good reasons for examining any of a great number of anthropogenic chemicals. The work group assumed that the project advisory group or other workshops would deal with these specific questions before initiating the long-term program. Part of Phase II efforts should concentrate on refinements of Phase I methods. Ongoing field verification and validation are important aspects of a program to demonstrate the utility of the biomonitoring concept.

An area of particular relevance for understanding efficacy of bees as environmental samplers is an understanding of mass transports and cycling of pollutants from the environment to bees. Drs. R. Ferenbaugh and E. Gladney, Los Alamos Scientific Laboratory, are interested in establishing bee colonies in a greenhouse large enough that they can be maintained without being allowed to forage outside. The bees would be supplied with water and/or sugar solution spiked with radioisotope tracers. Up-take of these tracers and subsequent partitioning within the hive as well as effects on the colonies would be monitored. For elements such as arsenic that have no useable radioisotopes, studies on non-radiactive istopes could be conducted.

A similar line of investigation is currently being carried out at North Central Bee Research Laboratory, Wisconsin, where they are looking at electrical charges on bees and materials such as pesticides and how the charges affect the likelihood of contaminants being transported back to the colonies.

Both of these research areas attempt to explain more clearly how materials are moved from the environment onto and through bee systems - invaluable information if bees are to be used as samples of their surroundings.

PHASE II - EFFECTS MONITORING

The effects of the uptake and partitioning of contaminants by bee colonies will be another means of linking effects and exposure monitoring.

Although ecosystems warrant a high priority for Phase II, the transfer of mutagenic, carcinogenic, or toxic substances not only from the environment into bee colonies but also through hives and/or pollen to human food chains was a major concern of several of the work group members. Exploratory research aimed at hazardous chemicals in bee systems, which have never been looked at before, more or less obligates us to look at the potential for their transfer to humans.

Many of the Phase I activities are conceptually coupled using the honey bee population simulation model. Phase II emphasizes a cooperative effort with the Bioenvironmental Bee Laboratory to adapt the model to pollutant impacts and to conduct field tests of the sensitivity of the various bee parameters that control it. The model appears to have a high potential for use as a research tool for predicting the impact of pollutants.

During Phase II, the emphasis will continue to be at the level of the colony, although at the level of the individual bee there are concerns that should be addressed. Given the priority of examining effects to bee systems and ecosystems, the types of measurements which might be made at the colony level include; foraging activity, honey crops, survival of colonies in a given ecosystem, and possibly requirements for adaptive bee management to offset pollutant effects such as the need for more sugar syrup, the use of pollen substitutes, or movement of colonies. On the level of the individual it may be useful to examine: predisposition to diseases, shifts in microflora, changes in longevity, or alterations in physiology.

The obvious link to the pollination syndrome is the numbers of foragers available. Pollutant effects on colony foraging activity will depress floral reproductive success. However, the native bee populations are also a component of the pollination subsystem which needs to be assessed. This portion of the effort constitutes Phase III.

PHASE III

Phase III activities (Table 5) do not necessarily begin during the third year. The emphasis of Phases I and II are on the honey bee and the work plans are ambitious, even with strong financial support during the second year. Studies of effects at the ecosystem levels, particularly as regards the pollination syndrome, will be labor intensive. Phase III sets out to develop Passive Monitors using native bees and to better address the ecosystem level (pollination competition between native and domestic bees). If Phase III could be started along with Phase II, so much the better.

Essentially, Phase III consists of following the same steps to develop native bees for use as monitors as were taken for honey bees. Hopefully, not every test will have to be repeated, but native bees may respond differently. The native bees are much more susceptible to many pesticide residues than honey bees.

TABLE 5. RECOMMENDED BEE RESEARCH PROJECTS

PHASE III	
EXPOSURE MONITORING	EFFECTS MONITORING
Field and Laboratory Studies o Passive Monitors --*Osmia, Lassioglossus* --Bioaccumulation --Sampling Variability --Correlations of Pollutant Levels in Bees and Environment o Field Validations of Monitoring Techniques Derived from Phases I and II	Continuation and Expansion of Phase II Studies Concerning Impacts to Honey Bee Systems and to Ecosystems Field and Laboratory Studies of Passive Monitors o Dose-response Tests --Population Dynamics --Reproductive Cycles --Physiological Responses o Adaptation and Testing of Megachilidae Simulation Model o Validation of Effects to Pollination Systems

Native bees also remove some of the problems associated with the "management" of the bees. Also, native bees are a structural and functional part of the ecosystem in which they occur, while honey bees are often inserted and may in some ways be out of context.

Currently, there is a Megachilidae model being developed at Oregon State University. Hopefully, it will be available by Phase III and can be used in much the same manner as the honey bee simulation model.

Phase III, in addition to developing the use of native bees as passive monitors to compliment honey bees as active monitors, will continue to develop, test and validate the honey bee monitoring methods which should be applicable to small scale network test by this time.

PHASE IV

After passive and active monitoring tools have been proven in the laboratory and adapted to the field, their use can be extended (Table 6) to actual monitoring efforts. At this time, site selection becomes a major issue. Regional or national networks require sites that provide a representative cross-section of major ecosystems, including both those relatively undisturbed (wilderness areas) and disturbed agricultural and urban regions.

TABLE 6. RECOMMENDED BEE RESEARCH PROJECTS

PHASE IV	
EXPOSURE MONITORING	EFFECTS MONITORING
o Expansion to localized or Regional Monitoring Networks o Bibliographies Relevant to Methods, Chemicals, Bees as Exposure Monitors.	Field Validations of Effects Monitoring Techniques for Active and Passive Monitors.

SECTION 5

MONITORING THE SOILS MEDIUM

This report outlines a research package demonstrating the utility of the concept of network biological monitoring through development of specific tools. The adopted strategy focuses on species identified in earlier workshops as the most promising biological monitors of the soils media - earthworms and aims at:

- o Correlating toxic material trends in the media with body burden trends (exposure monitoring).
- o Identifying possible biological responses to those trends (effects monitoring).
- o Gaining insight into possible physiological mechanisms by which these responses may be brought about.

In addition, the soils work group felt strongly that this monitoring of important ecological components should be coupled with monitoring of important functional processes such as nutrient flux. Given limited resources in the first year and uncertain resources in succeeding years, the soils work group has developed a phased program potentially leading to implementation of a national network at the end of four years.

5.1 PHASE I - LABORATORY AND PAPER STUDIES DEMONSTRATING "PROOF OF CONCEPT"

There can be little doubt about the need for a capability to monitor accumulations and effects of man-induced pollutants. To date, however, efforts in this direction have mostly involved theorizing rather than research to develop the tools. The soils work group believes that even one demonstration of a workable technique might well release a virtual flood of support for further development. Thus, "proof" of the utility of the conceptual framework already developed is a necessary first step in developing an effective monitoring network within that framework. The group gathered at this workshop represents a bringing together, perhaps for the first time, of the expertise on earthworm life cycles, bioaccumulation, toxic effects and neurophysiology, as well as expertise in ecological and soil sciences, needed to provide reasonable assurance of success.

<u>Rationale</u>

Even given this assurance, the extensive debate of preceeding workshops suggests a need to express the assumptions under which Phase I research is believed to fit the requirements and address the concerns of monitoring network theory. A nonprioritized listing of these assumptions is as follows:

- The approach recommended does not have to be the only or even the best approach for the soil medium, only a way that our collective experience suggests will work.

- The initial effort should be as focused as is possible, while still—covering enough bases to promise a useable product. Thus, it may be adequate to identify only one or a few species with the potential for accumulating one or more chemicals likely to cause measurable effects that have significance to man and his environment.

- Soil components and processes are extremely important as monitoring candidates because:

  -- Soil is the medium in terrestrial ecosystems which controls productivity.

  -- Soil is the ultimate depository/repository of most pollutants reaching terrestrial ecosystems.

  -- Decomposition of organic matter returning basic nutrients to the soil is a critical point of convergence in terrestrial ecosystem functioning. Decomposition processes may therefore, provide an excellent index to ecosystem health.

  -- While many studies of pollutant effects upon ecosystem level processes (e.g. nutrient cycling) exist, it is difficult to monitor accumulation in soils without a biological model that can act as an exposure model.

- Previous workshops and work groups' experience suggests that body burdens of trace metals in earthworms, particularly accumulations of cobalt 60 may be the best point of beginning. Earthworms are one of the most important organisms responsible for mechanical mixing of the soil and play a major role in maintaining physical soil characteristics and processes such as aeration, water permeability, and mineral turnover (Van Hook, 1974). Earthworms are key components in natural food chains providing a food source for many small mammals and birds. Earthworms have been demonstrated to exert a significant effect on redistribution of cadmium, carbon, and cesium in soils. Due to this redistribution effect and the earthworm's ubiquitous occurrence in nature, these invertebrates may exert a significant influence on the distribution of trace elements in soils and in food chains by altering concentrations in tissues through bioaccumulation (Van Hook, 1974). The earthworm provides the only logical monitor for exposure (if not effects) in soils at this stage because:

  -- Distribution growth requirements and life history are known.

  -- Earthworms can be worked with in both the laboratory and field.

-- Their physiology and taxonomy has been characterized.

-- They are large enough to permit segregation and analysis of tissues.

-- They have a defined structural importance in terrestrial ecosystems and at least a conceptually significant functional role (that is mixing, microbial turnover, trophic transfer).

-- A partial data base exists linking physiology, population dynamics, and some soil processes.

o There is a general principle that the explanation for an observed phenomemon (such as a population crash) must be sought at the next lower level of organization (lower egg production) and its significance is best appreciated at the next higher level of biological organization (community structure). An effective monitoring network must provide information ranging from trends in pollutant concentrations for environmental media, and resulting body burdens, all the way to biological effects and their significance at the ecosystem level of organization. Consequently, a research approach which provides integrated data from several levels of organization, in both structural and functional terms, would represent an optimum approach.

o Earthworms appear to meet the above criteria in several ways:

-- They are one of the only organisms that is sufficiently large and widely distributed to offer quantities of experimental material at a variety of locations.

-- They are proven accumulators.

-- Lethal and sublethal responses to environmental exposures and tissue accumulations have been demonstrated.

-- The techniques for relating organism and population responses to physiological mechanisms and to ecosystem function (such as nutrient flux in the soil column) are available.

-- If causative mechanisms can be shown in earthworm neuromuscular physiology at the cellular level, the results may have broad applications to other animals.

-- We may be able to relate effects on structure (earthworm population) to effects on function (nutrient cycling).

o At the same time there are some limitations to be kept in mind:

-- Earthworms are found only in certain types of soils and their use in the passive mode is limited to regions where such soils occur.

-- The relationships between environmental exposure and body burdens will not be simple ones. For example, distribution of pollutants in the soil medium does not necessarily equal worm exposure due to variations in, for example, sorption on clays, chelation and microbial exclusion.

— Changes from the laboratory (nutrient enriched) to the field (nutrient normal) will radically affect the above exposure variables as well as process rates (microbial activity, for example) and will make direct extrapolations to the field difficult.

-- Laboratory experiments initially focusing on effects to a single age class must eventually address populations with normal age distributions before the move to passive monitoring in the field can be made.

-- Even with the experience available in the work group, there are too many unknowns to permit moving with confidence directly into the field. The strategy must be to first "proof" the concept in the laboratory before moving to the field.

-- Field populations subjected to pollutant stress over long periods may accomodate or adapt to ambient concentrations and therefore fail to respond in the ways laboratory studies would lead us to expect. Thus, once laboratory experiments have demonstrated the utility of the approach, it must be validated in the field for both active monitoring (standard organisms and soils placed in the field and their responses to pollution gradients measured) and passive monitoring (organisms and soils sampled in the field along existing pollution gradients).

Testing the Earthworm as a Biomonitor

Given the above advantages and constraints, the expertise available in the work group was applied to laboratory experiments investigating the feasibility of using earthworms as a biomonitor. Design of these experiments is based on a simplified conceptual model (Figure 5) of key interactions between the earthworm, *Eisenia foetida*, and its environment. Our observations indicate that *E. foetida* is primarily a microvore that, under field conditions, feeds in concentrated organic sources such as manure. Organic matter is consumed along with microbes; however, sterile organic matter cannot support the worms. Thus, accumulation of heavy metals is assumed to be primarily the results of consumption at a single trophic level. However, a simple interpretation is complicated by (1) "recycling" of heavy metals by microbial uptake from feces and (2) possible cuticular uptake of metals obtained from the substrate (indicated by dashed flow from manure to the worms). The former problem will be partially controlled and the substrate levels of heavy metals maintained at near-steady state levels by placing the worms in a new food source every two weeks.

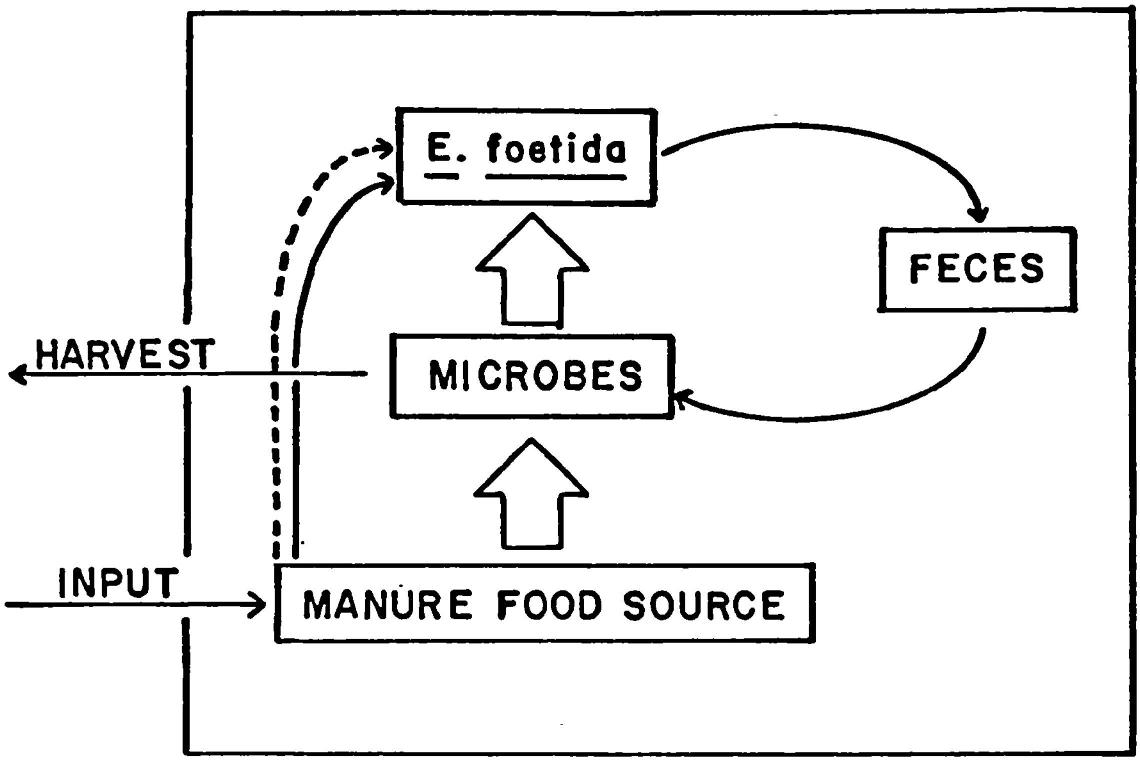

Figure 5. Conceptual model of key interactions between the earthworm, *Eisenia foetida*, and its environment.

Exposure, Bioaccumulation and Population Effects--

The easiest and most relevant parameters to measure in earthworms regarding biological effects monitoring would be growth, reproduction and neural functioning. The species of worm we propose to use in the study would be *Eisenia foetida*, due to the large amount known about this worm. The following research plan is designed to show how these parameters can be measured.

Recent work shows that growth and reproduction of earthworms can be used as a model system. Preliminary evidence has demonstrated that they both accumulate and are sensitive to heavy metals, with regards to growth and reproduction.

Initial screening will be done with five levels of heavy metals within a broad range such as 0-10,000 ppm. The material to be tested will be mixed with the food source. The earthworms used in this procedure will all be newly hatched worms (<6mg) and mortality and weight changes will be determined bi-weekly for 8 weeks. A narrower range of heavy metals will be selected which represents levels from minimal effects to nearly lethal dosages.

Five levels using sublethal concentrations can now be tested for growth rates and total body burdens. Total body burden would be done by digesting the worm and assaying the heavy metals using AAS.

In addition, long-term growth and reproductive studies, (24 weeks), would be carried out using sublethal concentrations of each heavy metal. Rate of cocoon production would be an indication of the stress placed upon a population due to the heavy metals.

Neural Effects--

The monitoring of neurophysiological effects caused by heavy metals is desirable because nervous system function is known to be greatly impaired by such substances and because the earthworm's nervous system control the animal's locomotory behavior in the soil.

Recent work has demonstrated the electrical activity arising from identifiable neurons, as well as from associated muscle units can be easily detected with an array of extracellular recording electrodes in contact with the ventral surface of completely intact earthworms. Such an approach offers a unique advantage for non-invasive and long-term monitoring of neurophysiological parameters. Given this advantage it should be possible to obtain a detailed assessment of impairment of neural function caused by the presence of known amounts of heavy metals.

Animals will be reared at the same five sublethal levels of heavy metal described in the previous section on exposure versus bioaccumulation and effects. Neurophysiological parameters to be examined on a bi-weekly basis include: (1) action potential conduction velocity in single nerve fibers, (2) effectiveness of central nervous system synaptic transmission, and (3) effectiveness of neuromuscular synaptic transmission. From such experiments we can establish dose-response relationships for each of the neurophysiological parameters. In addition, we can identify specific loci and modes of action of the metals within the nervous system.

Our understanding of the impairment of neural function by toxicant accumulation in the earthworm may have relevance to and lead to a better understanding of the adverse neural effects of toxicants on other soil invertebrates, which are not as amenable to neurophysiological study. In addition these studies could yield insight into general mechanisms of cellular-level action of heavy metals of vertebrate, as well as invertebrate nervous systems. Finally, and perhaps most important, the impairment of neural and/or muscle function by heavy metals could be a critical factor in modifying ecologically significant locomotor activities of the animal, such as escape withdrawal from predators or rates of burrowing in the soil.

<u>Using Soil Nutrient Flux as a Functional Monitor</u>

Rationale--

Biological monitoring at the species or population level alone allows for generalizations to be directed at only one level of ecological organization above that studied. The use of an earthworm population as a biomonitor allows us to make inferences on the structure of the litter-soil community. Inferences to the functioning of this community are tenuous at best due, in part, to potential compensatory mechanisms of biota under perturbed conditions (e.g. Edwards, 1965). At the same time, however, there exists an ample and expanding data base to suggest that the direct links between a dominant structural component of an ecosystem or subsystem and functional characteristics of the system are strongly correlated and predictable, at least over an "ecological" time frame.

Ecosystem level behaviors are not just the sum of subsystem behaviors but also the result of interactions between subsystem components. Thus, the measurement of an ecosystem property includes not only the effects of our biological monitor - in this case the dominant soil invertebrate of many economically important systems - but also the monitor's interactions with other components of the system. Validation of biological monitoring is therefore contingent upon establishing the functional attributes of the monitor within an ecosystem perspective and requires, at least initially, concurrent analysis of the functional response of the litter-soil system to those same conditions experienced by the biomonitor.

Concept Paper Synthesizing Current Knowledge--

Limited resources available during Phase I may preclude experimentation with a functional monitor (nutrient flux) in parallel with that on the biomonitor (earthworm) even though such pairing would be ideal (perhaps saving a year in implementation of a monitoring network). However, much of the research necessary to demonstrate the utility of such a pairing has already been done. A highly useful Phase I exercise would be to produce a concept paper synthesizing current knowledge on the use of soil nutrient flux as an ecological monitor of pollution. Examples of the material useful to this state-of-the-art summary follow:

- Early papers by O'Neill, Reichle and Shugart showing that, theoretically, nutrient cycling processes are extremely sensitive to stress and are potentially the primary functional compensatory mechanisms to stress.

- Water, Air, Soil Pollution. 1977 O'Neill *et al.*
  Describes laboratory and field experiments showing ecological responses to heavy metal pollution; results in all cases were that nutrient loss increased at a lower dose than microbial or invertebrate physiological or population parameters changed.

- Series of papers from Mehillo, Vitusek on Hubbard Brook, Cromack and Sollins on Coniferous forests, and Waide at Georgia supporting theory.

- Series of papers showing functional stability measures compared to traditional diversity stability measures.

- Series of lab and field experiments using As, Cu, Cd, Pb, hexachlorobenzene, fluidized-bed effluents, phenols, fly ash, uranium mill tailings, strontium, plutonium - most measure populations, physiological microbial indices as well as nutrient export.

Thus, the purpose of the concept paper would be to furnish (1) a state-of-art review of a terrestrial "ecomonitor," and (2) a statement of the advantages of coupling this with a biomonitor (such as coupling trends in environmental pollution with trends in tissues, physiological and population effects, and measuring partial contribution to effects noted at the ecosystem level.) This paper would therefore provide a theoretical test of the hypothesis that an active monitoring system using (1) a biomonitor (e.g. earthworm) to address exposure and at least first generation effects -- on physiological and population parameters and (2) and ecomonitor (e.g. litter-soil cores) to address functional (e.g. nutrient-cycling) effects on the ecosystem would provide an adaptable approach to short-term and long-term characterization of a site under baseline or perturbed conditions.

Phase I Cost Estimates

The following are very rough minimal estimates for each of three activities over the course of one calendar year:

Exposure, Bioaccumulation and Population Effects

1.	analysis of heavy metal by AA (1000 samples)	10,000
2.	salaries - one full-time technician	12,500
3.	supplies	2,000
4.	publication costs	250
5.	travel - includes one trip to Iowa St. and one scientific meeting	1,000
6.	indirect costs -100% of salary above	12,500
		38,250

Neural Effects

1.	salaries - one half-time research assistant one half-time technician	6,250
2.	hourly labor - approve 6 hours/week at min. 3.10/hour	6,250
3.	equipment - oscilloscope, with amplifiers	3,500
	environmental chambers - 2@ 1000 ea	2,000
4.	publication costs	250
5.	travel -includes one trip to Syracuse and one science meeting	1,000

6.	supplies - photographic, electrical, chemical, glassware	2,500
7.	indirect costs - 70% of salaries above	8,750
		31,500

Concept Paper

1.	salaries	7,500
2.	clerical and publication	1,000
3.	indirect costs	7,500
		16,000

Net Cost	85,750

PHASE II - LABORATORY STUDIES CORRELATING BIOLOGICAL AND FUNCTIONAL SOIL MONITORS

The concept paper suggested for Phase I would be the primary basis for designing Phase II laboratory studies. These studies would employ the same materials used for a food source or contaminant at the same concentrations of pollutants used for earthworms, this time as input to an intact litter-soil system. Experiments would monitor the output of CO_2, DOC, PO_4-P or NH_3-N, NO_3-N as a function of dose. Efforts could be made at this time to look at other pollutants such as airborne hydrocarbons and pollutant interactions. Sensitivity of this system could be compared against effects on earthworms as a basis for extending the laboratory work to field situations.

PHASE III - FIELD STUDIES DEMONSTRATING COMBINED BIOLOGICAL/FUNCTIONAL MONITORS IN ACTIVE AND PASSIVE MODES

The laboratory and paper studies of Phase I and II will provide the data base for designing Phase III studies adapting the combined biological and functional monitors to field conditions in both active and passive modes. Although the details of this research must await the data base, there exists sufficient information to offer a skeleton design and research plan, (Table 7) and to suggest a promising site. Because the adaptation from laboratory to field is more direct for the active mode, it will be given the most attention here.

TABLE 7. PHASE III FIELD STUDY EXPERIMENTAL DESIGN

Passive Monitoring	Active Monitoring
o Sampling of litter and soil along environmental gradient: element analysis o Sampling and collection of earthworm species along gradient: -- Element analysis -- Neurophysiological testing	o Collection of litter and soil samples along gradient: removal to laboratory -- Introduce laboratory species of worm in half of samples - exposure analyses - effects analyses -- Functional analysis of samples with and without earthworms on other half of samples. Measurement of nutrient flux through samples.

Exposure, Bioaccumulation and Population Effects

Development of the active dual monitor would involve going to a test site and analyzing for heavy metals as well as physiological effects. This would give the ambient level of heavy metals found in the native population and the corresponding physiological impact.

The soil from this field site would then be tested with the same species of worms found in the field site but using worms that would not have been exposed to the high levels of heavy metals found in the test site. These "clean" worms could then be grown in the test site soils and later analyzed for heavy metal content as well as physiological effects.

This experimental design would then give a correlation between the amount of a contaminant found in a soil and (1) the amount found in a worm over a long and short exposure period and (2) physiological state of the animal. This would be a method of judging how well laboratory experiments correlate with field conditions.

Since the first phase of this research plan focuses on obtaining a data base for organismal and population effects of heavy metals on a single earthworm species, we propose to further expand our biological data base to include a number of different earthworm species representing a variety of natural geographical and soil horizon distributions. Some of these species include: *Allolobophora longa, A. turgida* and *Lumbricus ribellus*. Such studies would provide baseline information regarding species variation in heavy metal effects on growth, reproduction and physiological parameters. The general methods for carrying these studies have been described in the initial phase.

Proposed Site

Selection of a site for evaluating the proposed active and passive bio- and ecomonitoring system is extremely important to the overall success of this research. To illustrate this importance we considered the advantages of a previously studied lead smelter on the Crooked Creek watershed near Ralla, Missouri. The smelter has been in operation for almost 15 years emitting detectable amounts of Pb, Cu, Ck, Zn and Cd. This site was intensively studied under a NSF research grant (Rann) during 1971 to 1974 on vegetation, litter dynamics and hydrology. Models are available for plume dispersion, loading, and hydrological transport. This was the site of initial studies leading to many papers sited in the state-of-the-art review (Phase I). It would allow field evaluations to be done along a gradient which has a known history and could be quickly determined chemically at a new time. Earthworms are established at the site and would allow bonus determination of (1) accumulation (2) adaptive physiology over the hundreds of generations exposed, and (3) maximal concentrations of metals which can be adapted to by the genetic potential of the populations or their food sources.

Since much of the ecosystem theory suggesting nutrient export as a monitor of ecosystem level response to stress came from this area, a bonus would be the reevaluation of the theory's prediction. The prediction is that when the site is reevaluated, if the metal inputs have remained, that each effect would be measurable at greater distances than in the early 1970's. The importance of this is that, if proven, it shows that an ecomonitor (e.g. nutrient cycling) is a predictive indicator of structural changes in the system as a result of stress continuing over a finite time period.

Thus, the series of experiments outlined offers a way of:

- Evaluating the combined biological and functional monitoring tools.
- Demonstrating population and physiological responses to short-term perturbations (in the active mode) and long-term perturbations (in the passive mode).
- Measuring the relative sensitivities and adaptive resilience of both monitors over short and long time frames.

PHASE IV - APPLICATION TO A NATIONAL NETWORK

Once these monitoring tools have been proven in the laboratory and adapted to the field at a specific site their use may be broadened as part of a national or even an international monitoring network. Because earthworms are not found everywhere, some substitute that serves a comparable role in soil litter breakdown may have to be sought in arid and other extreme environments. The functional monitor proposed will likely serve at all sites.

Given the necessary tools, selection of appropriate sites becomes the next important hurdle. Current thinking (Luepke, 1979) suggests a need for a minimum set of monitoring sites that would provide a representative cross section of major ecosystems of concern. Such a network should include areas