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ABSTRACT

This course is intended for personnel who have an operational or administrative responsibility for the design and use of bioassay and biomonitoring, and who have no experience in conducting static bioassays. The training consists of classroom discussions, laboratory exercises and demonstrations, and demonstration and observation activities. (CO)

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Bioassay for Toxic and Hazardous Materials

Training Manual

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Bioassay for Toxic and Hazardous Materials

This course is for personnel who have an operational or administrative responsibility for the design and use of bioassay and biomonitoring, and who have no experience in conducting static bioassays.

After successfully completing the course, the student will have application knowledge about the most commonly accepted practices and principles involved in the laboratory use of aquatic organisms to detect or evaluate toxic and hazardous materials. He/she will be able to select, design, and operate a bioassay conforming to the current edition of Standard Methods for the Examination of Water and Wastewater.

The training consists of classroom discussions, laboratory exercises and demonstrations, and demonstration and observation at the Newtown Fish Toxicology Laboratory which employs bioassay and related techniques.

U. S. ENVIRONMENTAL PROTECTION AGENCY
Office of Water Program Operations
National Training and Operational Technology Center

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CONTENTS

THE DEVELOPMENT OF WATER QUALITY CRITERIA IN THE UNITED STATES

The genesis of water quality criteria in the United States can be traced to the early 1900's. Marsh,[1] in 1907, published on the effects of industrial wastes on fish. Shelford,[2] in 1917, published effect data on fish for a large number of gas-waste constituents. In this early publication he reiterated that the toxicity of waste differs for different species of fish and generally is greater for the smaller and younger fish. Powers,[3] working with Shelford, experimented with the goldfish as a test animal for aquatic toxicity studies.

A monumental early effort to describe and record the effects of various concentrations of a great number of substances on aquatic life was that of Ellis[4] in 1937. Ellis reviewed the existing literature for 114 substances and in a 72-page document listed lethal concentrations found by the various authors. He provided a rationale for the use of standard test animals in aquatic bioassay procedures and used the common goldfish, Carassius auratus, and the entomostracan, Daphnia magna, as test species on which experiments were made in constant-temperature cabinets.

Early efforts to summarize knowledge concerning water quality criteria took the form of a listing of the concentration, the test organism, the results of the test within a time period, and the reference for a cause-effect relationship for a particular water contaminant. In early bioassay efforts insufficient attention was given to the quality of the dilution water used for the experiment and to the effects of such dilution water on the relative toxicity of the tested contaminant. As a result, conclusions from citations of such references were, at best, difficult to formulate and most often were left to the discretion of the reader.

In 1952, the State of California[5] published a 512-page book on "Water Quality Criteria" that contained 1,369 references. This classic reference summarized water quality criteria promulgated by State and interstate agencies as well as the legal application of such criteria. Eight major beneficial uses of water were described. Three hundred pages of the document were devoted to cause-effect relationships for major water pollutants. The concentration-effect levels for the pollutant in question were discussed for each of the designated water used.

The State of California's 1952 "Water Quality Criteria" was expanded and tremendously enhanced into a second edition edited by Jack E. McKee and Harold W. Wolf and published in 1963 by the Resources Agency of California, State Water Quality Control Board.[6] This edition, which included 3,827 cited references, was a monumental effort in bringing together under one cover the world's literature on water quality criteria. Criteria were identified and referenced for a host of water quality characteristics according to their effects on domestic water supplies, industrial water supplies, irrigation waters, fish and other aquatic life, shellfish culture, and swimming and other recreational uses. Specific concentrations were arranged in ascending order indicating the degree of damage to fish in the indicated time and under the conditions of exposure. The results of such a tabulation presented a range of values and, as would be expected by those investigating such conditions, there was often an overlap in values among those concentrations that had been reported as harmful by others. Such an anomaly is due to differences in investigative techniques among investigators, the characteristics of the water used as a diluent for the toxicant, the physiological state of the test organisms, and variations in the temperature in which the tests were conducted. Nevertheless, the tabulation of criteria values for each of the water quality constituents has been helpful in predicting a range within which a water quality constituent would have a deleterious effect upon the receiving waterway.

In 1966 the Secretary of the Interior appointed a number of nationally recognized scientists to a National Technical Advisory Committee to develop water quality criteria for five specified uses of water: agricultural, industrial, recreational, fish and wildlife, and domestic water supply. In 1968 the report was published.[7] This report constituted the most comprehensive documentation to date on water quality requirements for particular and defined water uses. The book was intended to be used as a basic reference by personnel in state water pollution control agencies engaged in water quality studies and water quality standards setting activities. In some respects, this volume represented a marriage between the best available experimental or investigative criteria recorded in the literature and the judgments of recognized water quality experts with long experience in associated management practices. Its publication heralded a change in the concept of water quality criteria from one that listed a series of concentration-effect levels to another that recommended concentrations that would ensure the protection of the quality of the aquatic environment and the continuation of the designated water use. When a specific aquatic life recommendation for a particular water pollutant could not be made because of either a lack of information or conflicting information, a recommendation was made to substitute a designated application factor based upon data obtained from a 96-hour bioassay using a sensitive aquatic organism and the receiving water as a diluent for the toxicity test.

The U.S. Environmental Protection Agency contracted with the National Academy of Sciences and the National Technical Advisory Committee's "Water Quality Criteria" and to develop a water quality criteria document that would include current knowledge. The result was a 1974 publication that presented water quality criteria as of 1972.[8]

The Federal Water Pollution Control Act Amendments of 1972 (P. L. 92-500) mandated that the Environmental Protection Agency publish water quality criteria accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water.

Section 304(a) of P. L. 92-500 states:

(1) The Administrator, after consultation with appropriate Federal and State agencies and other interested persons, shall develop and publish, within one year after the date of enactment of this title (Oct. 18, 1972) (and from time to time thereafter revise) criteria for water quality accurately reflecting the latest scientific knowledge (A) on the kind and extent of all identifiable effects on health and welfare including, but not limited to, plankton, fish, shellfish, wildlife, plant life, shorelines, beaches, aesthetics, and recreation which may be expected from the presence of pollutants in any body of water, including ground water; (B) on the concentration and dispersal of pollutants, or their byproducts, through biological, physical, and chemical processes; and (C) on the effects of pollutants on biological community diversity, productivity, and stability, including information on the factors affecting rates of eutrophication and rates of organic and inorganic sedimentation for varying types of receiving waters.

(2) The Administrator, after consultation with appropriate Federal and State agencies and other interested persons, shall develop and publish, within one year after the date of enactment of this title (Oct. 18, 1972) (and from time to time thereafter revise) information (A) on the factors necessary to restore and maintain the chemical, physical, and biological integrity of all navigable waters, ground waters, waters of the contiguous zone, and the oceans; (b) on the factors necessary for the protection and propagation of shellfish, fish, and wildlife for classes and categories of receiving waters and to allow recreational activities in and on the water; and (C) on the measurement and classification of water quality; and (D) for the purpose of Section 303 of this title, on and the identification of pollutants suitable for maximum daily load measurement correlated with the achievement of water quality objectives.

(3) Such criteria and information and revisions thereof shall be issued to the States and shall be published in the Federal Register and otherwise made available to the public.

Section 101(a)(2) of P. L. 92-500 states:

It is the national goal that wherever attainable, an interim goal of water quality which provides for the protection and propagation of fish, shellfish, and wildlife, and provides for recreation in and on the water be achieved by July 1, 1983..."

The objectives of this volume are to respond to these sections of the Act and thus establish water quality criteria. The "Quality Criteria for Water" will be expanded periodically in the future to include additional constituents as data become available. While the NAS/NAE "1972 Water Quality Criteria" considered aluminum, antimony, bromine, cobalt, fluoride, lithium, molybdenum, thallium, uranim, and vanadium, these presently are not included in this volume; however, they should be given consideration in the development of Statewater quality standards and quality criteria may be developed for them in future volumes of the QCW. In particular geographical areas or for specific water uses such as the irrigation of certain crops, some of these constituents may have harmful effects. Until such time that criteria for the 10 aforementioned constituents are developed, information relating to their effects on the aquatic ecosystem may be found in the NAS/NAE "1972 Water Quality Criteria."

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DESCRIPTORS:

Bioassay, Methodology, Water Quality Criteria

This outline was extracted from: Quality Criteria for Water - 1976. The Development of Water Quality Criteria in the United States

THE PHILOSOPHY OF QUALITY CRITERIA

Water quality criteria specify concentrations of water constituents which, if not exceeded, are expected to result in an aquatic ecosystem suitable for the higher uses of water. Such criteria are derived from scientific facts obtained from experimental or in situ observations that depict organism responses to a defined stimulus or material under identifiable or regulated environmental conditions for a specified time period.

Water quality criteria are not intended to offer the same degree of safety for survival and propogation at all times to all organisms within a given ecosystem. They are intended not only to protect essential and significant life in water, as well as the direct users of water, but also to protect life that is dependent on life in water for its existence, or that may consume intentionally or unintentionally any edible portion of such life.

The criteria levels for domestic water supply incorporate available data for human health protection. Such values are different from the criteria levels necessary for protection of aquatic life. The Agency's interim primary drinking water regulations (40 FR 59566 Dec. 24, 1975), as required by the Safe Drinking Water Act (42 U.S.C. 300f, et seg.), incorporate applicable domestic water supply criteria. Where pollutants are identified in both the quality criteria for domestic water supply and the Drinking Water Standards, the concentration levels are identical. Water treatment may not significantly affect the removal of certain pollutants.

What is essential and significant life in water? Do Daphnia or stonefly nymphs qualify as such life? Why does 1/100th of a concentration that is lethal to 50 percent of the test organisms (LC_{50}) constitute a criterion in some instances, whereas 1/20 or 1/10th of some effect levels constitutes a criterion in other instances? These are questions that often are asked of those who undertake the task of criteria formulation.

The universe of organisms composing life in water is great in kind and number. As in the human population, physiological variability exists among individuals of the same species in response to a given stimulus. A much greater response variation exists among species of aquatic organisms. Thus, aquatic organisms do not exhibit the same degree of harm, individually or by species, from a given concentration of a toxicant or potential toxicant within the environment. In establishing a level or concentration of a quality constituent as a criterion it is necessary to ensure a reasonable degree of safety for those more sensitive species that are important to the functioning of the aquatic ecosystem even though data on the response of such species to the quality constituent under consideration may not be available. The aquatic food web is an intricate relationship of predator and prey organisms. A water constituent that may in some way destroy or eliminate an important segment of that food web would, in all likelihood, destroy or seriously impair other organisms associated with it.

Although experimentation relating to the effects of particular substances under controlled conditions began in the early 1900's, the effects of any substance on more than a few of the vast number of aquatic organisms have not been investigated. Certain test animals have been selected by investigators for intensive investigation because of their importance to man, because of their availability to the researcher, and because of their physiological responses to the laboratory environment. As general indicators of organism responses such test organisms are representative of the expected results for other associated organisms. In this context Daphnia or stoneflies or other associated organisms indicate the general levels of toxicity to be expected among untested species. In addition, test organisms are themselves vital links within the food web that results in the fish population in a particular waterway.

The ideal data base for criteria development would consist of information on a large percentage of aquatic species and would show the community response to a range of concentrations for a tested constituent during a long time period. This information is not available but investigators are beginning to derive such information for a few water constituents. Where only 96-hour bioassay data are available, judgmental prudence dictates that a substantial safety factor be employed to protect all life stages of the test organism in waters of varying quality, as well as to protect associated organisms within the aquatic environment that have not been tested and that may be more sensitive to the test constituent. Application factors have been used to provide the degree of protection required. Safe levels for certain chlorinated hydrocarbons and certain heavy metals were estimated by applying an 0.01 application factor to the 96-hour LC_{50} value for sensitive aquatic organisms. Flow-through bioassays have been conducted for some test indicator organisms over a substantial period of their life history. In a few other cases, information is available for the organism's natural life or for more than one generation of the species. Such data may indicate a minimal effect level, as well as a no-effect level.

The word "criterion" should not be used interchangeably with, or as a synonym for, the work "standard." The word "criterion" represents a constituent concentration or level associated with a degree of environmental effect upon which scientific judgment may be based. As it is currently associated with the water environment it has come to mean a designated concentration of a constituent that when not exceeded, will protect an organism, an organism community, or a prescribed water use or quality with an adequate degree of safety. A criterion, in some cases, may be a narrative statement instead of a constituent concentration. On the other hand a standard connotes a legal entity for a particular reach of waterway or for an effluent. A water quality standard may use a water quality criterion as a basis for regulation or enforcement, but the standard may differ from a criterion because of prevailing local natural conditions, such as naturally occurring organic acids, or because of the importance of a particular waterway, economic considerations, or the degree of safety to a particular ecosystem that may be desired.

Toxicity to aquatic life generally is expressed in terms of acute (short term) or chronic (long term) effects. Acute toxicity refers to effects occurring in a short time period; often death is the end point. Acute toxicity can be expressed as the lethal concentration for a stated percentage of organisms tested, or the reciprocal, which is the tolerance limit of a percentage of surviving organisms. Acute toxicity for aquatic organisms generally has been expressed for 24- to 96-hour exposures.

Chronic effects often occur in the species population rather than in the individual. If eggs fail to develop or the sperm does not remain viable, the species would be eliminated from an ecosystem because of reproductive failure. Physiological stress may make a species less competitive with others and may result in a gradual population decline or absence from an area. The elimination of a microcrustacean that serves as a vital food during the larval period of a fish's life could result ultimately in the elimination of the fish from an area. The phenomenon of bioaccumulation of certain materials may result in chronic toxicity to the ultimate consumer in a food chain. Thus, fish may mobilize lethal toxicants from their fatty tissues during periods of physiological stress. Egg shells of predatory birds may be weakened to a point of destruction in the nest. Bird chick embryos may have increased mortality rates. There may be a hazard to the health of man if aquatic organisms with toxic residues are consumed.

The fact that living systems, i.e., individuals, populations, species and ecosystems can take up, accumulate, and bioconcentrate manmade and natural toxicants is well documented. In aquatic systems biota are exposed directly to pollutant toxicants through submersion in a relatively efficient solvent (water) and are exposed indirectly through food webs and other biological, chemical, and physical interactions. Initial toxicant levels, if not immediately toxic and damaging, may accumulate in the biota or sediment and increase to levels that are lethal or sublethally damaging to aquatic organisms or to consumers of these organisms. Water quality criteria reflect a knowledge of the capacity for environmental accumulation, persistence, and effects of specific toxicants in specific aquatic systems.

Ions of toxic materials frequently cause adverse effects because they pass through the semi-permeable membrances of an organism. Molecular diffusions through membranes may occur for some compounds such as pesticides, polychlorinated biphenyls and other toxicants. Some materials may not pass through membranes in their natural or waste-discharged state, but in water they may be converted to states that have increased ability to affect organisms. For example; certain microorganisms can methylate mercury, thus producing a material that more readily enters physiological systems. Some materials may have multiple effects; for example, an iron salt may not be toxic; an iron floc or gel may be an irritant or clog fish gills to effect asphyxiation; iron at low concentrations can be a trace nutrient but at high concentrations it can be toxicant. Materials also can affect organisms if their metabolic byproducts cannot be excreted. Unless otherwise stated, criteria are based on the total concentration of the substance because an ecosystem can produce chemical, physical, and biological changes that may be detrimental to organisms living in or using the water.

In prescribing water quality criteria certain fundamental principles dominate the reasoning process. In establishing a level or concentration as a criterion for a given constituent it was assumed that other factors within the aquatic environment are acceptable to maintain the integrity of the water. Interrelationships and interactions among organisms and their environment, as well as the interrelationships of sediments and the constituents they contain to the water above, are recognized as fact.

Antagonistic and synergistic reactions among many quality constituents in water also are recognized as fact. The precise definition of such reactions and their relative effects on particular segments of aquatic life have not been identified with scientific precision. Historically, much of the data to support criteria development was of an ambient concentration-organism response nature. Recently, data are becoming available on long term chronic effects on particular species. Studies now determine carcinogenic, teratogenic, and other insidious effects of toxic materials.

Some unpolluted waters in the Nation may exceed designated criteria for particular constituents There is variability in the natural quality of water and certain organisms become adapted to that quality which may be considered extreme in other areas. Likewise, it is recognized that a single criterion cannot identify minimal quality for the protection of the integrity of water for every aquatic ecosystem in the Nation. To provide an adequate degree of safety to protect against long term effects may result in a criterion that cannot be detected with present analytical tools. In some cases, a mass balance calculation can provide a means of assurance that the integrity of the waterway is not being degraded.

Water quality criteria do not have direct regulatory impact, but they form the basis for judgment in several Environmental Protection Agency programs that are derived from water quality considerations. For example, water quality standards developed by the States under Section 303 of the Act and approved by EPA are to be based on the water quality criteria, appropriately modified to take account of local conditions. The local conditions to be considered include actual and projected uses of the water, natural background levels of particular constituents, the presence or absence of sensitive important species, characteristics of the local biological community, temperature and weather, flow characteristics, and synergistic or antagonistic effects of combinations of pollutants.

Similarly, by providing a judgment on desirable levels of ambient water quality, water quality criteria are the starting point in deriving toxic pollutant effluent standards pursuant to Section 307(a) of the Act. Other EPA programs that make use of water quality criteria include drinking water standards, the ocean dumping program, designation of hazardous substances, dredge spoil criteria development, removal of in-place toxic materials, thermal pollution, and pesticide registration.

To provide the water resource protection for which they are designed, quality criteria should apply to virtually all of the Nation's navigable waters with modifications for local conditions as needed. To violate quality criteria for any substantial length of time or in any substantial portion of a waterway may result in an adverse effect on aquatic life and perhaps a hazard to man or other consumers of aquatic life.

Quality criteria have been designed to provide long term protection. Thus, they may provide a basis for effluent standards, but it is not intended that criteria values become effluent standards. It is recognized that certain substances may be applied to the aquatic environment with the concurrence of a governmental agency for the precise purpose of controlling or managing a portion of the aquatic ecosystem; aquatic herbicides and piscicides are examples of such substances. For such occurrences, criteria obviously do not apply. It is recognized further that pesticides applied according to official label instructions to agricultural and forest lands may be washed to a receiving waterway by a torrential rainstorm. Under such conditions it is believed that such diffuse source inflows should receive consideration similar to that of a discrete effluent discharge and that in such instances the criteria should be applied to the principal portion of the waterway rather than to that peripheral portion receiving the diffuse inflow.

The format for presenting water quality criteria includes a concise statement of the dominat criterion or criteria for a particular constituent followed by a narrative introduction, a rationale that includes justification for the designated criterion or criteria, and a listing of the references cited within the rationale. An effort has been made to restrict supporting data to those which have either been published or are in press awaiting publication. A particular constituent may have more than one criterion to ensure more than one water use or condition, i.e., hard or soft water where applicable, suitability as a drinking water supply source, protection of human health when edible portions of selected biota are consumed, provision for recreational bathing or water skiing, and permitting an appropriate factor of safety to ensure protection for essential warm or cold water associated biota.

Criteria are presented for those substances that may occur in water where data indicate the potential for harm to aquatic life, or to water users, or to the consumers of the water or of the aquatic life, or to water users, or to the consumers of the water or of the aquatic life. Presented criteria do not represent an all-inclusive list of constituent contaminants. Omissions from criteria should not be construed to mean that an omitted quality constituent is either unimportant or nonhazardous.

DESCRIPTORS:

Bioassay, Water Quality Criteria

This outline was extracted from: Quality Criteria for Water - 1976.

SIGNIFICANCE OF "LIMITING FACTORS" TO POPULATION VARIATION

I INTRODUCTION

A All aquatic organisms do not react uniformly to the various chemical, physical and biological features in their environment. Through normal evolutionary processes various organisms have become adapted to certain combinations of environmental conditions. The successful development and maintenance of a population or community depend upon harmonious ecological balance between environmental conditions and tolerance of the organisms to variations in one or more of these conditions.

B A factor whose presence or absence exerts some restraining influence upon a population through incompatibility with species requirements or tolerance is said to be a limiting factor. The principle of limiting factors is one of the major aspects of the environmental control of aquatic organisms (Figure 1).

II PRINCIPLE OF LIMITING FACTORS

This principle rests essentially upon two basic concepts. One of these relates organisms to the environmental supply of materials essential for their growth and development. The second pertains to the tolerance which organisms exhibit toward environmental conditions.

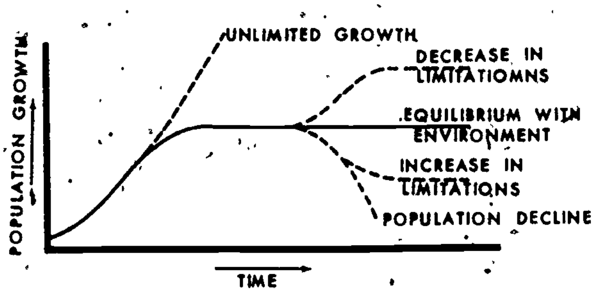

Figure 1. The relationships of limiting factors to population growth and development.

A Liebig's Law of the Minimum enunciates the first basic concept. In order for an organism to inhabit a particular environment, specified levels of the materials necessary for growth and development (nutrients, respiratory gases, etc.) must be present. If one of these materials is absent from the environment or present in minimal quantities, a given species will only survive in limited numbers, if at all (Figure 2). Copper, for example, is essential in trace amounts for many species.

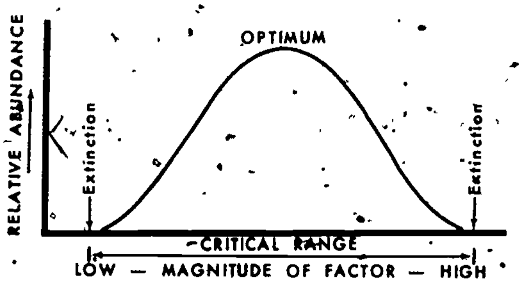

Figure 2. Relationships of environmental factors and the abundance of organisms.

1 The subsidiary principle of factor interaction states that high concentration or availability of some substance, or the action of some factor in the environment, may modify utilization of the minimum one. For example:

a The uptake of phosphorus by the algae Nitzchia closterium is influenced by the relative quantities of nitrate and phosphate in the environment; however, nitrate utilization appears to be unaffected by the phosphate (Reid, 1961).

b The assimilation of some algae is closely related to temperature.

c The rate of oxygen utilization by fish may be affected by many other substances or factors in the environment.

d Where strontium is abundant, mollusks are able to substitute it, to a partial extent, for calcium in their shells (Odum, 1959).

2 If a material is present in large amounts, but only a small amount is available for use by the organism, the amount available and not the total amount present determines whether or not the particular material is limiting (calcium in the form of $CaCO_3$).

B Shelford pointed out in his Law of Tolerance that there are maximum as well as minimum values of most environmental factors which can be tolerated. Absence or failure of an organism can be controlled by the deficiency or excess of any factor which may approach the limits of tolerance for that organism (Figure 3).

Minimum Limit of Toleration		Range of Optimum of Factors	Maximum Limit of Toleration	
Absent	Decreasing Abundance	Greatest Abundance	Decreasing Abundance	Absent

Figure 3. Shelford's Law of Tolerance.

1 Organisms have an ecological minimum and maximum for each environmental factor with a range in between called the critical range which represents the range of tolerance (Figure 2). The actual range thru which an organism can grow, develop and reproduce normally is usually much smaller than its total range of tolerance.

2 Purely deleterious factors (heavy metals, pesticides, etc.) have a maximum tolerable value, but no optimum (Figure 4).

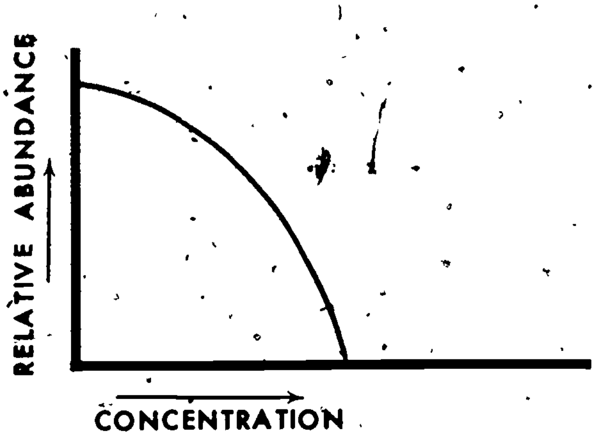

Figure 4. Relationship of purely harmful factors and the abundance of organisms.

3 Tolerance to environmental factors varies widely among aquatic organisms.

a A species may exhibit a wide range of tolerance toward one factor and a narrow range toward another. Trout, for instance, have a wide range of tolerance for salinity and a narrow range for temperature.

b All stages in the life history of an organism do not necessarily have the same ranges of tolerance. The period of reproduction is a critical time in the life cycle of most organisms.

c The range of tolerance toward one factor may be modified by another factor. The toxicity of most substances increases as the temperature increases.

d The range of tolerance toward a given factor may vary geographically within the same species. Organisms that adjust to local conditions are called ecotypes.

e The range of tolerance toward a given factor may vary seasonally. In general organisms tend to be more sensitive to environmental changes in summer than in other seasons. This is primarily due to the higher summer temperatures.

4 A wide range of distribution of a species is usually the result of a wide range of tolerances. Organisms with a wide range of tolerance for all factors are likely to be the most widely distributed, although their growth rate may vary greatly. A one-year old carp, for instance, may vary in size from less than an ounce to more than a pound depending on the habitat.

5 To express the relative degree of tolerance for a particular environmental factor the prefix <u>eury</u> (wide) or <u>steno</u> (narrow) is added to a term for that feature (Figure 5).

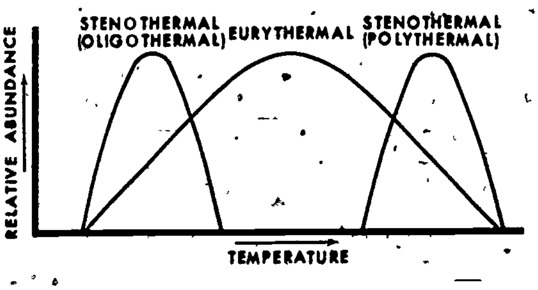

Figure 5. Comparison of relative limits of tolerance of stenothermal and eurythermal organisms.

C The law of the minimum as it pertains to factors affecting metabolism, and the law of tolerance as it relates to density and distribution, can be combined to form a broad principle of limiting factors.

1 The abundance, distribution, activity and growth of a population are determined by a combination of factors, any one of which may through scarcity or overabundance be limiting.

2 The artificial introduction of various substances into the environment tends to eliminate limiting minimums for some species and create intolerable maximums for others.

3 The biological productivity of any body of water is the end result of interaction of the organisms present with the surrounding environment.

III VALUE AND USE OF THE PRINCIPLE OF LIMITING FACTORS

A The organism-environment relationship is apt to be so complex that not all factors are of equal importance in a given situation; some links of the chain guiding the organism are weaker than others. Understanding the broad principle of limiting factors and the subsidiary principles involved make the task of ferreting out the weak link in a given situation much easier and possibly less time consuming and expensive.

1 If an organism has a wide range of tolerance for a factor which is relatively constant in the environment that factor is not likely to be limiting. The factor cannot be completely eliminated from consideration, however, because of factor interaction.

2 If an organism is known to have narrow limits of tolerance for a factor which is also variable in the environment, that factor merits careful study since it might be limiting.

.3

B Because of the complexity of the aquatic environment, it is not always easy to isolate the factor in the environment that is limiting a particular population. Premature conclusions may result from limited observations of a particular situations. Many important factors may be overlooked unless a sufficiently long period of time is covered to permit the factors to fluctuate within their ranges of possible variation. Much time and money may be wasted on control measures without the real limiting factor ever being discovered or the situation being improved.

C Knowledge of the principle of limiting factors may be used to limit the number of parameters that need to be measured or observed for a particular study. Not all of the numerous physical, chemical and biological parameters need to be measured or observed for each study undertaken. The aims of a pollution survey are not to make and observe long lists of possible limiting factors but to discover which factors are significant, how they bring about their effects, the source or sources of the problem, and what control measures should be taken.

D Specific factors in the aquatic environment determine rather precisely what kinds of organisms will be present in a particular area. Therefore, organisms present or absent can be used to indicate environmental conditions. The diversity of organisms provides a better indication of environmental conditions than does any single species. Strong physio-chemical limiting factors tend to reduce the diversity within a community; more tolerant species are then able to undergo population growth.

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Descriptors: Population, Limiting Factors

GLOBAL ENVIRONMENTAL QUALITY

I FROM LOCAL TO REGIONAL TO GLOBAL PROBLEMS

A Environmental problems do not stop at national frontiers, or ideological barriers. Pollution in the atmosphere and oceans taints all nations, even those benignly favored by geography, climate, or natural resources.

1 The smokestacks of one country often pollute the air and water of another.

2 Toxic effluents poured into an international river can kill fish in a neighboring nation and ultimately pollute international seas.

B In Antarctica, thousands of miles from pollution sources, penguins and fish contain DDT in their fat. Recent layers of snow and ice on the white continent contain measurable amounts of lead. The increase can be correlated with the earliest days of lead smelting and combustion of leaded gasolines. PCB's are universally distributed.

C International cooperation, therefore, is necessary on many environmental fronts.

1 Sudden accidents that chaotically damage the environment - such as oil spills from a tanker at sea - require international cooperation both for prevention and for cleanup.

2 Environmental effects cannot be effectively treated by unilateral action.

3 The ocean can no longer be considered a dump.

D "One of the penalties of an ecological education is that one lives alone in a world of wounds. Much of the damage inflicted on land is quite invisible to laymen. An ecologist must either harden his shell and make believe that the consequences of science are none of his business, or he must be the doctor who sees the marks of death in a community that believes itself well and does not want to be told otherwise." Aldo Leopold

II CHANGES IN ECOSYSTEMS ARE OCCURRING CONTINUOUSLY

A Myriad interactions take place at every moment of the day as plants and animals respond to variations in their surroundings and to each other. Evolution has produced for each species, including man, a genetic composition that limits how far that species can go in adjusting to sudden changes in its surroundings. But within these limits the several thousand species in an ecosystem, or for that matter, the millions in the biosphere, continuously adjust to outside stimuli. Since interactions are so numerous, they form long chains of reactions.

B Small changes in one part of an ecosystem are likely to be felt and compensated for eventually throughout the system. Dramatic examples of change can be seen where man has altered the course of nature. It is vividly evident in his well-intentioned but poorly thought out tampering with river, lake, and other ecosystems.

1 The Aswan High Dam

2 The St. Lawrence Seaway

3 Lake Kariba

4 The Great Lakes

5 Valley of Mexico

6 California earthquake (Scientific American 3981, p. 333)

7 Everglades and the Miami, Florida Jetport

8 Copperhill, Tennessee (Copper Basin)

9 (You may add others)

C Ecosystem Stability

1 The stability of a particular ecosystem depends on its diversity. The more interdependencies in an ecosystem, the greater the chances that it will be able to compensate for changes imposed upon it.

2 A cornfield or lawn has little natural stability. If they are not constantly and carefully cultivated, they will not remain cornfields or lawns but will soon be overgrown with a wide variety of hardier plants constituting a more stable ecosystem.

3 The chemical elements that make up living systems also depend on complex, diverse sources to prevent cyclic shortages or oversupply.

4 Similar diversity is essential for the continued functioning of the cycle by which atmospheric nitrogen is made available to allow life to exist. This cycle depends on a wide variety of organisms, including soil bacteria and fungi, which are often destroyed by pesticides in the soil.

5 A numerical expression of diversity is often used in defining stream water quality.

D Biological Pollution

Contamination of living native biotas by introduction of exotic life forms has been called biological pollution by Lachner et al. Some of these introductions are compared to contamination as severe as a dangerous chemical release. They also threaten to replace known wildlife resources with species of little or unknown value.

1 Tropical areas have especially been vulnerable. Florida is referred to as "a biological cesspool of introduced life."

2 Invertebrates

a Asian Clams have a pelagic veliger larvae, thus, a variety of hydro installations are vulnerable to sub-sequent pipe clogging by the adult clams.

b Melanian snails are intermediate hosts for various trematodes parasitic on man.

3 Vertebrates

a At least 25 exotic species of fish have been established in North America.

b Birds, including starlings and cattle egrets.

c Mammals, including nutria.

4 Aquatic plants

Over twenty common exotic species are growing wild in the United States. The problem of waterway clogging has been especially severe in parts of the Southeast.

5 Pathogens and Pests

Introduction of insect pests and tree pathogens have had severe economic effects.

III LAWS OF ECOLOGY

A Four principles have been enunciated by Dr. Barry Commoner.

1 Everything is connected to everything else.

2 Everything must go somewhere.

3 Nature knows best.

4 There is no such thing as a free lunch.

B These may be summarized by the principle, "you can't do just one thing."

IV THE THREE PRINCIPLES OF ENVIRONMENTAL CONTROL (Wolman)

A You can't escape.

B You have to organize.

C You have to pay.

V LEOPOLD'S PRINCIPLE OF BIOTIC CAPITAL

"The releases of biotic capital tend to becloud or postpone the penalties of violence". Can you apply this to other parts of this outline?

VI POLLUTION COMES IN MANY PACKAGES

A The sources of ~~air~~, water, and land pollution are interrelated and often interchangeable.

1 A single source may pollute the air with smoke and chemicals, the land with solid wastes, and a river or lake with chemical and other wastes.

2 Control of air pollution may produce more solid wastes, which then pollute the land or water.

3 Control of wastewater effluent may convert it into solid wastes, which must be disposed of on land, or by combustion to the air.

4 Some pollutants - chemicals, radiation, pesticides - appear in all media.

B "Disposal" is as important and as costly as purification.

VII PERSISTENT CHEMICALS IN THE ENVIRONMENT

Increasingly complex manufacturing processes, coupled with rising industrialization, create greater amounts of exotic wastes potentially toxic to humans and aquatic life.

They may also be teratogenic (toxicants responsible for changes in the embryo with resulting birth defects, ex., thalidomide), mutagenic (insults which produce mutations, ex., radiation), or carcinogenic (insults which induce cancer, ex., benzopyrenes) in effect. Most carcinogens are also mutagenic. For all of these there are no threshhold levels as in toxicity. Fortunately there are simple rapid tests for mutagenicity using bacteria. Tests with animals are not always conclusive.

A Metals - current levels of cadmium, lead, and other substances are a growing concern for they affect not only fish and wildlife but ultimately man himself. Mercury pollution, for example, has become a serious problem, yet mercury has been present on earth since time immemorial.

B Pesticides

1 A pesticide and its metabolites may move through an ecosystem in many ways. Hard (pesticides which are persistent, having a long half-life in the environment includes the organochlorines, ex., DDT) pesticides ingested or otherwise borne by the target species will stay in the environment, possibly to be recycled or concentrated further through the natural action of food chains if the species is eaten. Most of the volume of pesticides do not reach their target at all.

2 Biological magnification

Initially, low levels of persistent pesticides in air, soil, and water may be concentrated at every step up the food chain. Minute aquatic organisms and scavengers, which screen water and bottom mud having pesticide levels of a few parts per billion, can accumulate levels measured in parts per million - a thousandfold increase. The sediments including fecal deposits are continuously recycled by the bottom animals.

a Oysters, for instance, will concentrate DDT 70,000 times higher in their tissues than it's concentration in surrounding water. They can also partially cleanse themselves in water free of DDT.

b Fish feeding on lower organisms build up concentrations in their visceral fat which may reach several thousand parts per million and levels in their edible flesh of hundreds of parts per million.

c Larger animals, such as fish-eating gulls and other birds, can further concentrate the chemicals. A survey on organochlorine residues in aquatic birds in the Cnadian prairie provinces showed that California and ring-billed gulls were among the most contaminated. Since gulls breed in colonies, breeding population changes can be detected and related to levels of chemical contamination. Ecological research on colonial birds to monitor the effects of chemical pollution on the environment is useful.

C "Polychlorinated biphenyls" (PCB's). PCB's are used in plasticizers, asphalt, ink, paper, and a host of other products. Action has been taken to curtail their release to the environment, since their effects are similar to hard pesticides.

D Other compounds which are toxic and accumulate in the ecosystem:

1 Phalate esters - may interfere with pesticide analyses

2 Benzopyrenes

E Refractory compounds like pentachlorophenal and hexachlorophene are poorly removed by both water treatment plants and wastewater treatment plants.

F It is estimated that 80% to 90% of cancers are caused by chemicals both in the working environment and total environment. This is shown by high risk industries and living areas.

G Most of the problems of persistent and dangerous chemicals in the environment are "after-the-fact". The solution obviously is tied to prevention. This is extremely complicated by economics, ignorance, and decision as to risks involved. Some advertising slogans now have more than an intended meaning.

H Wittingly or unwittingly we have all become a King Mithridates. And even a fish is no longer a fish!

VIII ACID RAIN

Acid rain is also becoming a problem in this country.

IX EXAMPLES OF SOME EARLY WARNING SIGNALS THAT HAVE BEEN DETECTED BUT FORGOTTEN, OR IGNORED.

A Magnetic micro-spherules in lake sediments now used to detect changes in industrialization indicate our slowness to recognize indicators of environmental change.

B Salmonid fish kills in poorly buffered clean lakes in Sweden. Over the past years there had been a successive increase of SO_2 in the air and precipitation. Thus, air-borne contamination from industrialized European countries had a great influence on previously unpolluted waters and their life.

C Minimata, Japan and mercury pollution.

D Organochlorine levels in commercial and sport fishing stocks, ex., the lower Mississippi River fish kills.

X SUMMARY

A Ecosystems of the world are linked together through biogeochemical cycles which are determined by patterns of transfer and concentrations of substances in the biosphere and surface rocks.

B Organisms determine or strongly influence chemical and physical characteristics of the atmosphere, soil, and waters.

C The inability of man to adequately predict or control his effects on the environment is indicated by his lack of knowledge concerning the net effect of atmospheric pollution on the earth's climate.

D Serious potential hazards for man which are all globally dispersed, are radionuclides, organic chemicals, pesticides, and combustion products.

E Environmental destruction is in lockstep with our population growth.

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Descriptors: Environmental Effects, Ecosystems

ECOLOGY PRIMER
(from Aldo Leopold's A SAND COUNTY ALMANAC)

I Ecology is a belated attempt to convert our collective knowledge of biotic materials into a collective wisdom of biotic management.

II The outstanding scientific discovery of the twentieth century is not television or radio, but rather the complexity of the land organism.

III One of the penalties of an ecological education is that one lives alone in a world of wounds. Much of the damage inflicted on land is quite invisible to laymen. An ecologist must either harden his shell and make believe that the consequences of science are none of his business, or he must be the doctor who sees the marks of death in a community that believes itself well and does not want to be told otherwise.

IV Ecosystems have been sketched out as pyramids, cycles, and energy circuits. The concept of land as an energy circuit conveys three basic ideas:

A That land is not merely soil.

B That the native plants and animals kept the energy circuit open; others may or may not.

C That man-made changes are of a different order than evolutionary changes, and have effects more comprehensive than is intended or foreseen (See figures 1-4).

D To keep every cog and wheel is the first precaution of intelligent tinkering.

V The process of altering the pyramid for human occupation releases stored energy, and this often gives rise, during the pioneering period, to a deceptive exuberance of plant and animal life, both wild and tame. <u>These releases of biotic capital tend to becloud or postpone the penalties of violence.</u>

VI A thing is right when it tends to preserve the integrity, stability, and beauty of the biotic community. It is wrong when it tends otherwise.

VII Every farm is a textbook on animal ecology; every stream is a textbook on aquatic ecology; conservation is the translation of the book.

VIII There are three spiritual dangers in not owning a farm

A One is the danger of supposing that breakfast comes from the grocery.

B Two is that heat comes from the furnace.

C And three is that gas comes from the pump.

IX In general, the trend of the evidence indicates that in land, just as in the fishes body, the symptoms may lie in one organ and the cause in another. The practices we now call conservation are, to a large extent, local alleviations of biotic pain. They are necessary, but they must not be confused with cures.

X An Atom at large in the biota is too free to know freedom; an atom back in the sea has forgotten it. For every atom lost to the sea, the prairie pulls another out of the decaying rocks. The only certain truth is that its creatures must suck hard, live fast, and die often, lest its losses exceed its gains.

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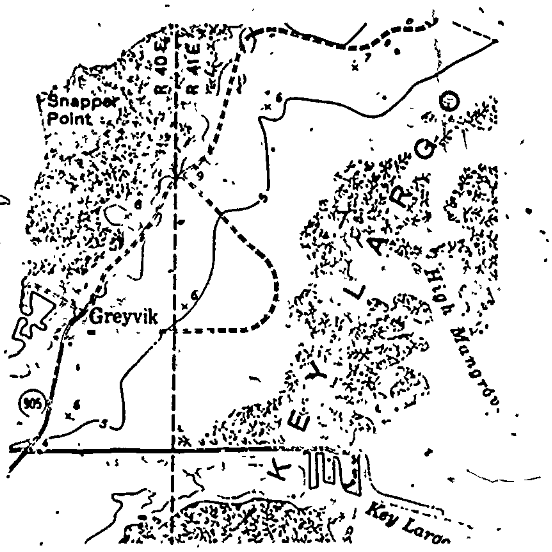
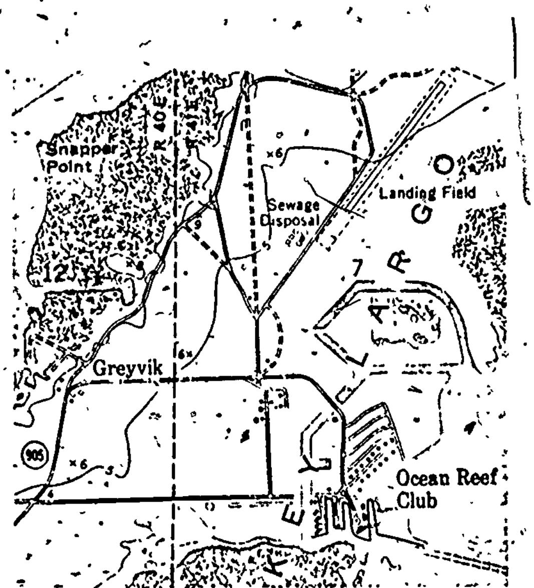
Descriptor: Ecology



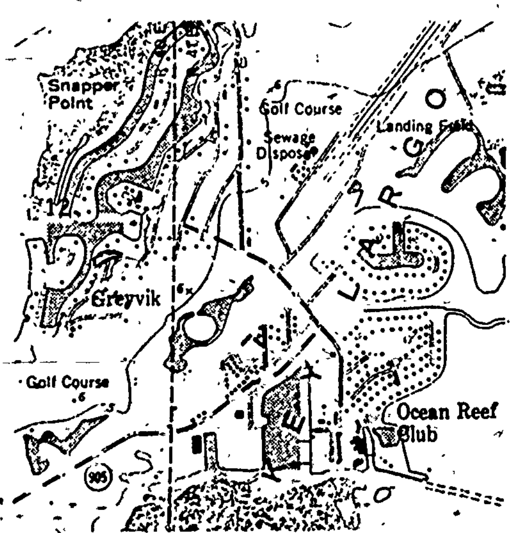
Mapped in 1949

Figure 1.



Revised in 1956

Figure 2.



Photorevised in 1969

Figure 3.

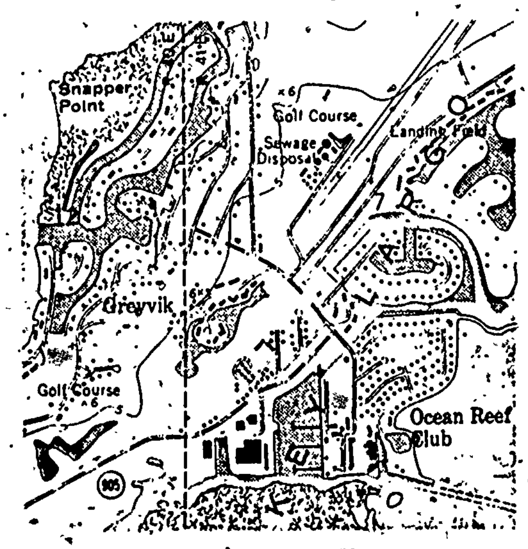


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Figure 4.

CLASSIFICATION OF COMMUNITIES, ECOSYSTEMS, AND TROPHIC LEVELS

I A COMMUNITY is an assemblage of populations of plants, animals, bacteria, and fungi that live in an environment and interact with one another, forming together a destinctive living system with its own composition, structure, environmental relations, development, and function.

II An ECOSYSTEM is a community and its environment treated together as a functional system of complementary relationships, and transfer and circulation of energy and matter. (A delightful litte essay on the odyssey of atoms X and Y through an ecosystem is in Leopold's, A Sand County Almanac.)

III TROPHIC levels are a convenient means of classifying organisms according to nutrition, or food and feeding. (See Figure 1.)

A PRODUCER, the photosynthetic plant or first organism on the food chain sequence. Fossil fuels were produced photosynthetically!

B Herbivore or primary CONSUMER, the first animal which feeds on plant food.

C First carnivore or secondary CONSUMER, an animal feeding on a plant-eating animal.

D Second carnivore or tertiary CONSUMER feeding on the preceding.

E Tertiary carnivore.

F Quaternary carnivore.

G DECOMPOSERS OR REDUCERS, bacteria which break down the above organisms. Often called the middlemen or stokers of the furnace of photosynthesis.

H Saprovores or DETRITIVORES which feed on bacteria and/or fungi.

I Macroinvertebrates have been subdivided into trophic levels according to feeding habits (See Figure 1 from Cummin's).

1 Collectors strain, filter, or otherwise collect fine particulate organic matter from the passing current.

2 Shredders feed on leaves, detritus, and coarse particulate organic matter.

3 Grazers feed on attached growths.

4 Predators feed on other organisms.

IV Taxonomic Groupings

A TAXOCENES, a specific group of organisms. Ex. midges. For obvious reasons most systematists (taxonomists) can specialize in only one group of organisms. This fact is difficult for the non-biologist to grasp!

B Size, which is often dictated by the investigator's sampling equipment and specific interests.

V Arbitrary due to organism habitat preferences, available sampling devices, personal preference of the investigator, and mesh sizes of nets and sieves.

A PLANKTON, organisms suspended in a body of water and at the mercy of currents. This group has been subject to numerous divisional schemes. Plants are PHYTOPLANKTON, and animals, ZOOPLANKTON. Those retained by nets are obviously, NET PLANKTON. Those passing thru even the finest meshed nets are NANNOPLANKTON.

B PERIPHYTON, the community of microorganisms which grow on submerged objects (substrates). Literal meaning "to grow around plants", however standard glass microslides are submersed in the aquatic habitat to standardize results.

C BENTHOS is often used to mean MACROINVERTEBRATES, although there are benthic organisms in other plant, animal, and protist groups. Benthic refers strictly to the bottom substrates of lakes, streams, and other water bodies.

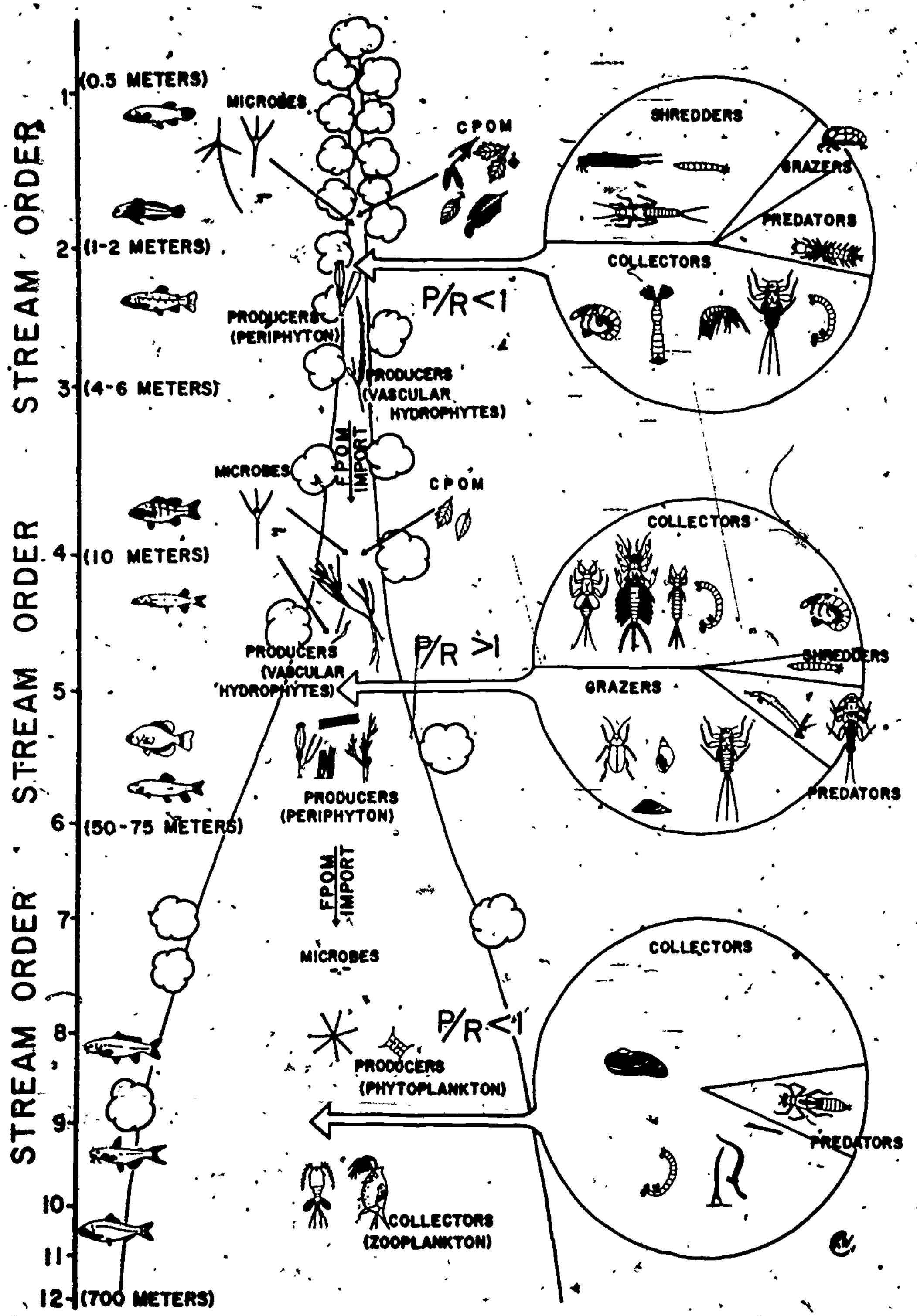
STREAM ORDER
STREAM ORDER
STREAM ORDER
1 (0.5 METERS)
2 (1-2 METERS)
3 (4-6 METERS)
4 (10 METERS)
5
6 (50-75 METERS)
7
8
9
10
11
12 (700 METERS)
MICROBES
CPOM
SHREDDERS
GRAZERS
PREDATORS
COLLECTORS
P/R<1
PRODUCERS (PERIPHYTON)
PRODUCERS (VASCULAR HYDROPHYTES)
FPOM IMPORT
MICROBES
CPOM
COLLECTORS
P/R >1
SHREDDERS
GRAZERS
PREDATORS
PRODUCERS (VASCULAR HYDROPHYTES)
PRODUCERS (PERIPHYTON)
FPOM IMPORT
MICROBES
COLLECTORS
P/R<1
PRODUCERS (PHYTOPLANKTON)
PREDATORS
COLLECTORS (ZOOPLANKTON)

FIGURE 1

D MACROINVERTEBRATES, are animals retained on a No. 30 mesh screen (approximately 0.5 mm) and thus visible to the naked eye.

E MACROPHYTES, the larger aquatic plants which are divided into emersed, floating, and submersed communities. Usually vascular plants but may include the larger algae and "primitive" plants. These have posed tremendous economic problems in the large man-made lakes, especially in tropical areas.

F NEKTON, in freshwater, essentially fish, salamanders, and the larger crustacea. In contrast to PLANKTON, these organisms are not at the mercy of the current.

G NEUSTON, or PLEUSTON, are inhabitants of the surface film (meniscus organisms), either supported by it, hanging from, or breaking through it. Other organisms are trapped by this neat little barrier of nature. The micro members of this are easily sampled by placing a clean cover slip on top of the surface film then either leaving it a specified time or examining it immediately under the microscope.

H DRIFT, macroinvertebrates which drift with the stream's current either periodically (diel or 24 hour), behaviorally, catastrophically or incidentally.

I BIOLOCIAL FLOCS, are suspended microorganisms that are formed by various means. In wastewater treatment plants they are encouraged in concrete aer aeration basins using diffused air or oxygen (the heart of the activated sludge process).

J MANIPULATED SUBSTRATE COMMUNITIES. Like the preceding community, theses are manipulated by man. Placing artificial or natural substrates in a body of water will cause these communities to appear thereon.

K We will again emphasize ARBITRARY, because organisms confound our neat little schemes to classify them. Many move from one community to another for various reasons. However, all these basic scheme do have intrinsic value, provided they are used with reason!

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Descriptors: Biological Communities

BIOASSAY AND BIOMONITORING

I INTRODUCTION

A An assay is an evaluation.

B A bioassay is an evaluation in which living organisms provide the scale.

1 The scale or degree of response may be the rate of growth or decrease of a population, colony, or individual; a behaviorial, physiological, or reproductive response, or simply a live or no-live response.

2 All types of bioassays may have a role to play in water quality evaluation at one time or another.

3 The particular group of bioassays discussed below are those which contribute to the evaluation of the effects of liquid wastes on aquatic environments in which experimental organisms such as fish are subjected to a series of concentrations of a known or suspected toxicant under adequately controlled conditions for a stipulated period of time.

C Historical Highlights

1 Prior to 1940, there was little or no uniformity in performing or reporting bioassays of water pollutants.

2 By mid-forties, the need for a standardized technique was becoming painfully obvious.

a The Atlantic Refining Company privately published the first statement of what is today, basically, our standard method (Hart, Doudoroff, and Greenbank, 1945).

b This was refined and accorded wide (but not universal) industrial, academic, and governmental acceptance in 1951 (Doudoroff, et al, 1951).

3 This method was first developed for the use of fishes, but has been found adaptable to a wide variety of organisms.

D Other Types or Plans of Bioassay

1 Many other designs for the expression of toxicity have been devised such as those based on time-concentration curves. Each has its advantages and proponents, but the basic Standard Method design remains the most widely used.

a In situ exposure of experimental organisms in cages or live cars, at selected sites above and below a suspected point or pollution is an obvious and time tested procedure, but lacks the precision of laboratory tests. It has the advantages of popular appeal, and of expressing actual environmental conditions.

b The familiar BOD test is a bioassay of the organic content of water subject to biodegradation.

E Biomonitoring

Water quality surveillance or monitoring by means of observing biota can be considered from two aspects: field and laboratory. It differs from bioassay primarily in the objective: a bioassay is an attempt to determine a specific defined value or threshold, whereas a biomonitoring operation is an attempt to use living organisms to ascertain whether or not aquatic life is endangered.

1 Periodic biological field surveys, samples, or other observation may demonstrate recent excessive pollution for example.

2 Organisms in a series of flow-through tanks in a laboratory may demonstrate the occurrence of an unacceptable

27

increase in the toxicity of an effluent, without measuring "how much" or "what."

II THE STANDARD METHOD BIOASSAY

A Introduction

1 This procedure is intended for use by industrial and other laboratories.

2 Its objective is to evaluate the toxicity of wastes and other water pollutants to fish or other aquatic organisms.

3 Potential applications are numerous.

a Dilution and/or treatment necessary to avoid acute toxic effects can be estimated.

b The efficacy of an existing treatment can be tested.

c The potential usefulness of a proposed treatment can be estimated.

4 The design of the test need not involve a chemical knowledge of the toxicant.

a Synergism, antagonism, and other interactions of chemical components cannot always be anticipated, but are automatically included in the result.

b All chemical and physical information available is, however, essential to the adequate interpretation and application of test results.

5 The test is best used for local application. Generalizations should be made with great caution.

6 Field observations should be made of results of application over a significant period of time.

7 Careful distinction should be made between fish mortality due to a physiological toxicant, and that due to lack of DO.

8 A uniform testing procedure is essential to effective action in water pollution control.

B Routine Procedure for Static Tests

1 Test organisms should be fish or other organisms of local significance.

a The most sensitive species available should be selected, but:

b They should be species which are amenable to captivity.

c They should be accurately identified.

d They should be relatively uniform in size. Individuals less than 3 inches in length are usually most convenient.

e They should be healthy and thoroughly acclimated to the laboratory.

f A careful record should be kept of their origin, handling, and condition.

2 Test water should preferably be taken from the receiving stream just above the discharge being evaluated or in a lake or estuary, beyond the influence of the discharge.

a If this is unsuitable, cleaner but similar waters from a more remote station may be substituted.

b Artificial "standard" waters are not recommended for general use, although many formulae have been proposed.

c In estuarine situations, a series of tests (marine grid) should be run, using waters of high and low salinities as characteristic of the region.

3 Other experimental conditions

a Temperature. The tests should be performed at a uniform temperature in the upper part of the expected summer range, e.g., 25 ± 2° C for warm water fish, and 15 ± 2° C for cold water species.

b Test containers should be of glass, widemouthed "pickle jugs" or battery jars are satisfactory. Five and one gallon sizes are both useful, but the larger size is required for conclusive results.

c Artificial aeration should not be used to maintain the dissolved oxygen concentration. If this falls below approximately 4 or 5 ppm at any time during the test, fewer fish should be used per container or an auxiliary oxygenation procedure invoked that is designed to avoid undue loss of volatile toxicants.

d The number of test animals should not be less than 10 per concentration for reliable conclusions; these may be distributed between two or more containers.

e Ratio of fish to solution. There should be not more than one gram of fish per liter of test solution.

4 Experimental procedure

a All dilutions for a given run should be prepared from the same sample.

b Duration. Tests should be run for at least 48 hours, preferable 96.

c Dead fish should be removed as soon as observed. Survivors should be counted and recorded each 24 hours.

d Feeding during the test should be avoided.

e Experimental concentrations. Any appropriate series of concentrations may be used. A logarithmic series such as is suggested in Table I is very convenient.

TABLE I

A Guide to the Selection of Experimental Concentrations, Based on Progressive Bisection of Intervals on a Logarithmic Scale.

Col. 1	Col. 2	Col. 3	Col. 4	Col. 5
10.0	----	----	----	----
----	----	----	----	8.7
----	----	----	7.5	----
----	----	----	----	6.5
----	----	5.6	----	----
----	----	----	----	4.9
----	----	----	4.2	----
----	----	----	----	3.7
----	3.2	----	----	----
----	----	----	----	2.8
----	----	----	2.4	----
----	----	----	----	2.1
----	----	1.8	----	----
----	----	----	----	1.55
----	----	----	1.35	----
----	----	----	----	1.15
1.0	----	----	----	----

Effluents of unknown, mixed, or variable composition are usually best expressed as percent by volume; while pure substances, or specific analyzable components are usually expressed as milligrams per liter (ppm). A control or reference tank containing dilution water only (with no toxicant) is essential, to demonstrate that all experimental organisms would have survived had it not been for the toxicant being tested.

f Expression of results. The measure of relative toxicity is the lethal concentration (symbol: LC). The time of exposure "t" must be shown along with the percentage of fish surviving (written as a postscript). For example, a 96 hour LC50 (optional: $LC50^{96\ hr}$) of a toxic substance is that concentration in which 50% of the experimental organisms survive for 96 hours. (Figure 1).

the critical range in acute toxicity

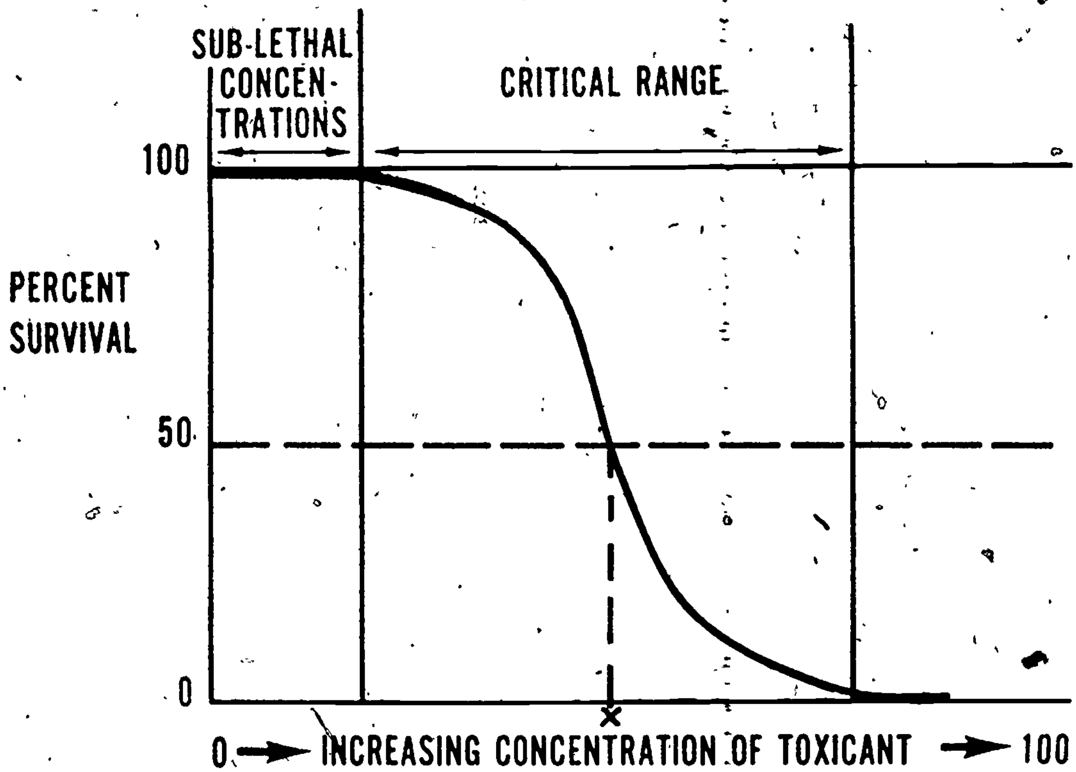

Figure 1
THE CRITICAL RANGE
X = LC50^t Concentration

1) A LC50^t is the equivalent of a median tolerance limit (TL_m^t).

2) This is analogous to the LC_{50} (concentration survived by 50% of the population) of the toxicologist, but is more universally usable with the parameters encountered in the water environment, some of which (such as temperature) cannot be expressed as "concentrations."

3) LC50's for 96 hours or less are arbitrarily referred to as measures of "acute" toxicity, while LC50's for longer periods of time are variously referred to as sub-acute, chronic, etc. (EC50 or effective concentration).

C Special Problems of Static Tests

1 Unaerated aquaria with finite quantities of toxicant are not always satisfactory (static tests).

a The toxicant may be volatile.

b Toxic materials may be masked by a high BOD.

c The toxicant may be progressively adsorbed on container walls, fish slime, metabolized or otherwise changed so that actual concentrations in tanks change with time.

2 Standards or requirements other than those involving toxicity per se may be involved.

3 Preliminary and concurrent investigations

a Obtain all available information about unknown to be tested.

b Does the material lend itself to this type of test?

c Run feasible on the spot analyses including DO.

d Significant quantities of solutions removed from test containers for analysis should be replaced with similar volume of same dilution.

4 Wastes with a high BOD or COD

a Suggested preliminary tests:

1) Set up two identical exploratory tests.

2) Aerate one but not the other.

3) If great difference develops between them, special procedures are indicated.

b Oxygenation or aeration of dilution water before making dilutions may help.

c Renewal of solutions at stated intervals (12, 24, or 48 hours) is approved. Fish are not harmed by being carefully transferred from one container to another. It is useful where:

1) Initial DO is adequate but slowly exhausted.

2) Toxicant is volatile, progressively adsorbed, precipitated, or otherwise changed.

D Continuous Flow Procedures

1 Continuous flow procedures imply the continuous or periodic renewal of the solutions in the experimental containers, at the same time maintaining the stated concentrations (including control). The variety of devices and flow plans to accomplish this are almost infinite, two general principals will be outlined below: assaying and monitoring.

2 Continuous flow bioassay, general advantages (Figure 2)

a Materials with moderate oxygen demands may be tested.

b Materials which degrade or are volatile may be tested.

c Due to the constant removal of metabolic and other wastes, and the constant supply of fresh oxygenated water, fish may be fed and so maintained over a longer period of time. Containers must of course be maintained in reasonably clean condition.

E Test Concentrations and End Points for Continuous Flow Assays

1 Test concentrations are in general less restricted than for static tests. They need not be so high as to insure achieving the desired end point in 48 or 96 hours, although they may be so set if desired.

2 Geometric type series of concentrations are still desirable (See Table 1).

3 Sub-lethal levels may be tested over entire life histories of organisms to determine long range effects.

4 In general, the setup should be prepared, calibrated and operated for several days, or until the concentrations have become chemically and physically stabilized before introducing the fish or other experimental organisms.

F Total Fish Weight and Liquid Volume in Continuous Flow Assays

In general, the constant renewal of test solution might appear to make possible testing more or larger fish in less water. Actually, flow-through volume and total weight of fish must be so related that adequate oxygen is maintained. Furthermore, over the longer periods of time involved, "lebensraum" (or territory) must be taken into account. Organisms must not be crowded to the extent that aggressive behavior and other ecological competitive factors are introduced.

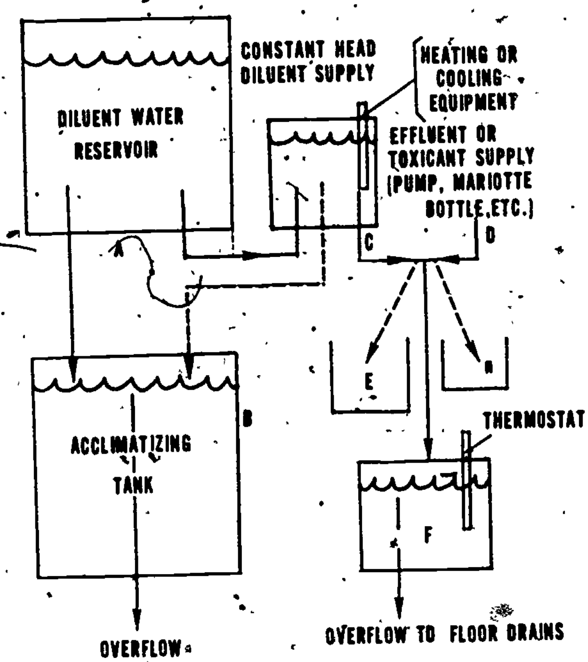

Figure 2

BASIC SETUP FOR CONTINUOUS FLOW BIOASSAY

E to n represents one of several exposure tanks containing a graded series of dilutions of the toxicant, including one control with none.

G End Points or Reactions to be Evaluated by Continuous Flow Assays

1 The original and traditional end point of biological evaluations such as those discussed here was the death of the organism. This was simple, direct, and usually unequivocal. Current practice, however, often involves much more sophisticated reactions such as reduction in the reporductive capacity, or a change in the breathing rate (movement of gills).

H Special Problems of Flow Through Bioassays

1 Due to the physical requirements of maintaining stated concentrations of chemicals over long periods of time, laboratory setups are usually complicated and always require attention and maintenance.

2 The problem of disease control frequently develops in populations held over a long period of time.

3 Water and/or power failure may jeopardize an assay experiment after months of time have been expended.

4 The expense of a long continued test may not be justified by the result.

5 The above points demonstrate that in general, flow through bioassays are not adapted to day-to-day routine toxicity determinations.

III REPORTING INTERPRETATION AND APPLICATION OF BIOASSAY RESULTS

A Reporting

1 Reports should include an orderly tabulation of all pertinent data such as:

a The type of setup used and duration of test

b Identity of experimental animals

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c Their source, history, average size and condition, and number used per concentration

d Source of, and chemical and physical analysis of experimental dilution water

e Experimental temperature.

f Volumes of experimental liquid in each container

g Records of routine analyses such as DO and pH

h Records of chemical analyses of toxicants in experimental tanks

i $LC50^t$ or other end point, and data from which it was determined.

B Interpretation and Application

1 The $LC50^t$ is an estimate of the mid-point of the critical concentration range the interval between the highest concentration at which all test animals survive, and the lowest at which they all die (Figure 1).

2 The final step is to extrapolate from this well established mid concentration to a safe concentration well below the "critical concentration range." Extrapolating or rather: "application factors" to accomplish this are still under development and will probably not be fully developed for many years. Available data indicate that these factors must be variable according to the toxicant in question acting in combination with the receiving water in question, and considering the entire aquatic community.

4 Other considerations

a Radioactive wastes must be evaluated with regard to their chemical toxicity as well as their radioactivity.

b Sub-acute levels of many toxicants such as lead, arsenic, cadmium, etc. may exert a low level chronic toxicity over a long period of time.

c "Safe levels" of a waste in regard to toxicity to aquatic life may still exceed standards of other types such as color, organic content, suspended solids, etc.

IV BIOMONITORING AS COMPARED TO BIOASSAY (Figure 3)

A Bioassay is (as stated above) the evaluation of the effects of stated concentrations of the test material for given periods of time.

B Biomonitoring is the use of organisms to detect change in an effluent (surveillance). It operates continuously and indefinitely.

C Bioassays typically involve relatively small flows and employ often especially prepared (perhaps repeatedly prepared) batches of experimental material, while biomonitoring typically involves larger flows, from operating industries or other installations.

D Bioassays basically determine:

1 Is the substance deleterious, and if so:

2 How deleterious is it?

E Biomonitoring is useful to

1 Demonstrate the continuous suitability of an effluent (or a predetermined dilution thereof) for the survival of the test organism.

2 Detect a change (usually deleterious) in the biological acceptability of the effluent.

3 To detect a change in the effect of a mixture of the effluent and the receiving water on the test organism (i. e. to detect a change in the receiving water).

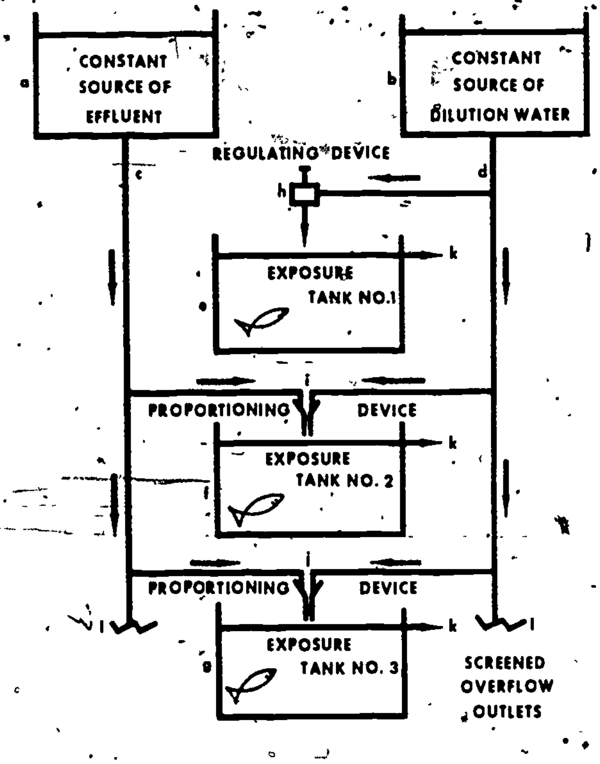

Figure 3

BASIC SETUP FOR BIOMONITORING

F Biomonitoring was originally effective only with relatively fast acting materials, or in situations where large changes might occur rather quickly (as for example, the accidental pickle liquor). Recent developments in the field of biotelemetry now make it feasible to "wire" a fish with electrodes (like the astronauts) and so to immediately record electronically any sudden or subtle change in the effluent which affects the physiological parameters being monitored on the live fish.

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Descriptors: Bioassay, Laboratory Equipment, Laboratory Tests

BIOASSAY FACILITIES AND EQUIPMENT

I INTRODUCTION

A Types of organisms and where they can be obtained are discussed elsewhere.

B Here we are concerned with facilities and equipment for working with the test animals.

II EXTENT OF FACILITIES AND EQUIPMENT NEEDS

A Depend on Several Considerations

1 Number and size of test animals

2 Type of study

a Static vs flow-through

b Death-survival vs autopsy-sublethal effects

c Laboratory setup vs outdoor setup

3 Space limitations

4 Budget and staff available or planned

5 Extent of bioassay program

III STATIC OR FLOW THROUGH

A Static Studies Suitable for:

1 Screening tests for "ball park" toxicity values to be used in long-term testing.

2 Comparative toxicity of compounds having similar metabolizing qualities.

3 Comparative toxicity of various process effluents in an industrial operation.

4 Screening organisms for relative sensitivity to a given toxicant.

5 When the available toxicant is in limited quantity it is sometimes necessary to use a static bioassay.

B Advantage of Static Test

1 Simplest to set up.

C Shortcomings of Static Test

1 Animals are bathed in their own waste materials, some of which are toxic.

2 Many toxicants decay with time, floc, or precipitate, resulting in lower than desired concentrations.

3 Some test organisms can absorb much of the toxicant into their tissues and reduce concentrations in water.

D Situations Which Should be Analyzed by Using a Flow Through Setup

1 LC determinations in general, exceptions only for extremely short-term tests.

2 Any test in which the size or hardiness of the test organisms compared to the volume of the test chamber suggests problems of waste products buildup or potential diminishing concentrations of toxicant.

3 Long-term tests studying effects of continuous or periodic exposures.

E Shortcomings of Flow-Through

1 Requires more space, time, and equipment.

F Advantage of Flow-Through

1 More accurate results.

IV NECESSARY FACILITIES AND EQUIPMENT

A For Static Bioassay

1 Discussed elsewhere

B For Flow Through Bioassay

1 Test chambers - glass, fiberglass, plastic, and stainless steel.

2 Dosage apparatus for adding toxicant, including gear pumps, constant-level-float siphons, Mariotte bottles, dipping bird gadgets, syringe devices, and various combinations of adjustable-volume venturi-siphon units.

3 Water flow control devices, including adjustable headboxes, constant level float valves, simple shut-off valves, capillary or tapered glass tubing, and adjustable-volume venturi-siphon units.

4 Combination units handling both toxicant and water.

a "Slurp-chamber" apparatus

b Serial diluters

c Simplified automatic dosage apparatus.

C For Special Application

1 Mount degasser

2 Temperature control devices and recorders

3 pH controlling unit and recorders

4 Demineralizers and carbon filters

5 Paddle wheel setup

6 Variety of test chambers

7 Swimming ability apparatus

8 Movement detectors and recorders

9 Egg collecting and hatching apparatus.

D Additional Items Needed or Useful

1 Necessities include: air pumps or compressed air system, plastic air tubing, glass and brass fittings for air tubing, air stones, small clamps, variety of plastic and rubber tubing, variety of regular and capillary glass tubing, rubber stoppers, formalin or alcohol preservative, chemical laboratory glassware (including pipets, graduate cylinders, etc.), fish-holding tanks for reserve specimens, small dip nets, food, data recording form stamps, pipet bulbs, water quality analyzing equipment (for DO, pH hardness, alkalinity, etc.), Toxicant analysis equipment (colorimeter, polarograph, chromatography setup), portable aerating equipment, fish and water transporting containers, fish treating compounds (antibiotics, parasite control chemicals), refrigeration facilities.

2 Items with special application: Dissecting equipment, tissue processing material (for fixing, embedding, and staining), microtome, microscopes, drying and ashing ovens, vacuum pump, scales and balance, activated carbon column, plankton counting equipment, appropriate text books and manuals.

E Space Requirements in General

1 Area for holding fish for future tests

2 Area for test chambers

3 Area for diluent water storage

4 Area for dosage apparatus

5 Area for water quality and toxicant analysis equipment

6 Sink and drainboard space

7 General storage area

8 Additional space for special equipment.

F Example of a Static Bioassay Laboratory (see Figure 1)

1 Area Number 1, provides space for holding and acclimatizing fish. Each

large aquarium for holding fish (A) is adequate for accommodating about 200-300 average size test specimens. An adequate air supply must be available at all times to provide for continuous aeration. Aquarium filters (C) of the inside type help in keeping the aquarium clean but best results come from continually trickling fresh water into the holding tank. Ordinary tap water can often be used for holding fish if chlorine has been removed, for example, by passing the water through an activated carbon column (E) and temperature changes are not too abrupt. The smaller aquarium (B) may be used for acclimating the test fish to the experimental water or holding them without food for period immediately preceding the tests.

2 Area Number 2 provides for storage and preparation of dilution water. While containers for hauling and storage may be of other inert materials, size, and shape, the polyethylene items illustrated (L, M) have been very satisfactory for this use. In addition

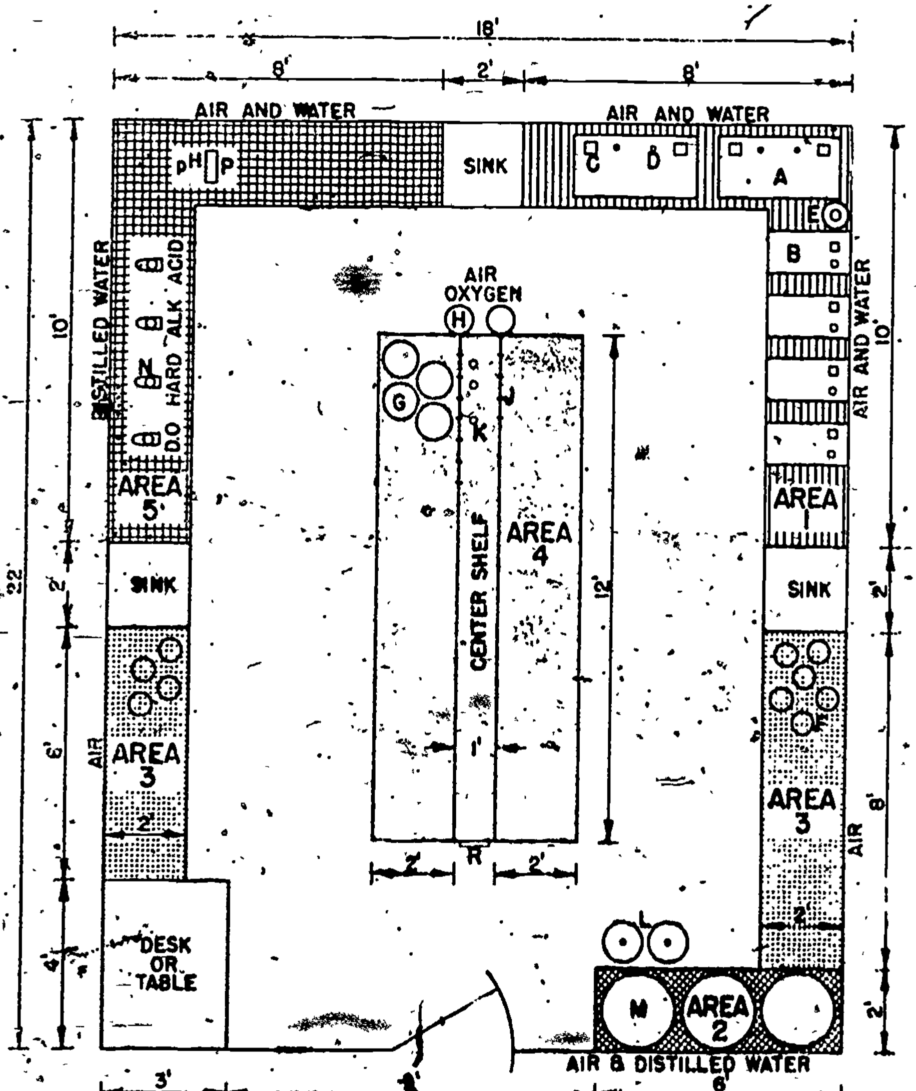

FIGURE 1

to the regular supply it may be desirable to have a supply of distilled or demineralized water.

3 Area Number 3 can be used for preparing experimental test concentrations of the effluent in the dilution water and for exploratory tests to determine the approximate toxic range. In these tests air may be needed, depending on the nature of the effluents.

4 Area Number 4 supplies bench space for holding 20 full-scale test chambers (G) on each side which permits the carrying on of at least 4 full-scale bioassays simultaneously. Air must be provided. A convenient arrangement for supplying air or oxygen is through a system of small tubing and 3-way air valves so that the supply to each test aquarium can be regulated independently. This system may be attached either to the air supply or to an oxygen cylinder (H) equipped with a pressure reduction valve and regulator.

5 Area Number 5 is for conducting the necessary chemical tests for oxygen control and to provide information necessary for interpreting bioassay data. Squeeze-o-matic burettes (N) have been found quite useful for rapid performance of certain chemical tests.

V DILUENT WATER

A Type of diluent water depends on the type of data sought.

1 Fresh, brackish, or salt water - depends on organisms.

2 If interested in the effect of a particular compound in a particular water body (stream, lake, estuary, etc.), then use water from the location where this compound would enter the waterway.

3 Generally for other purposes, the best water is the one you have most readily available. Tap water can be dechlorinated; spring, stream, or pond waters are usually suitable. Well water is not generally recommended, however.

4 By mixing water from two sources in large aerated storage facilities, close water quality can be maintained.

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Descriptors:

Bioassay, Laboratory Equipment

IMPORTANT DATA FROM ACUTE MORTALITY TESTS

I MEDIAN LETHAL CONCENTRATION (LC50)

A Mortality

The major result of an acute mortality test with a particular toxicant and a certain species of fish is the LC50, ie, the concentration that kills half of the fish. In order to calculate the LC50, one must know the percent mortality for a series of concentrations of the toxicant. Determining the percent mortality merely involves counting the live and dead fish, but one should report the criteria for determining whether a fish is live or dead.

B Concentration of Toxicant

Measuring the concentration of the toxicant is a more difficult problem. In some cases researchers do not measure the concentration of the toxicant. This is especially true of toxicity tests conducted with complex mixtures. In these cases, the results are calculated based on the amount of toxicant that was supposed to have been used. (The calculation of an LC50 from the mortality vs. concentration data will be covered in a later lecture).

In most cases the concentration of toxicant in the water is measured. However, it is not always easy to decide what measurement to make. Consider the following cases:

1 If a small amount of DDT is put into a jar of water about 70 percent will absorb to the glass walls of the jar, about 30 percent will accumulate at the air-water interface, and about 10 percent will dissolve in the water. Should one base an LC50 calculation on the total amount of DDT in the jar or on the concentration of DDT dissolved in the water?

2 If a moderate amount of copper sulfate is put into a jar of water, about 20 percent will dissolve and about 80 percent will become a basic copper precipitate. Some of the precipitate will form a scum on the water and the rest of it will be distributed throughout the water, probably with most of it on the bottom of the jar, depending on how well the fish stir up the water. Should one calculate the LC50 based on the total amount of copper in the jar, the dissolved copper, or the amount of copper dissolved and suspended in the water? The answer to this question must take into account the fact that some species of fish spend most of their time near the surface and some spend most of their time sitting on the bottom.

3 If phenol is placed in the water, it will exist both as unionized phenol and as ionized phenate ion. It is possible that one form is much more toxic than the other. However, practically all methods for measuring phenol in water will measure the total amount of phenol in the water.

These examples indicate that one must decide exactly how the sample must be taken and what analytical methods can be used. Very often the use of the results of the toxicity test will determine the answers to these questions. Usually for static bioassays the LC50 calculation is based on the total amount of toxicant put in the test container at the beginning of the test. This procedure obviously has its drawbacks. For continuous-flow tests, generally the best approach is to take a sample under the surface of the water. Because of the constant mixing in the test chamber, this usually, but not always, represents the total concentration of toxicant to which the fish are exposed.

The decision as to how the sample should be collected and/or what measurement should be made must depend on what question the test is supposed to answer. The two common questions are:

1. How much toxicant must be added to the water to affect the fish in a certain way?

2. How much toxicant must the fish be exposed to in order to affect them in a certain way?

C Calculation of the LC50

In reporting the results of a toxicity test one should report the LC50 for a given length of exposure with its confidence limits, the way the concentration of the toxicant was determined, and the method used to calculate the LC50 from the concentration-mortality data. Some people report the concentration-mortality data itself.

II OTHER INFORMATION

A About the Organism

There is much other information about an acute mortality test that should be reported along with the LC50 value. This can be broken down into four categories. One should report information about the fish, the test conditions, the toxicant, and the water used in the test. This information is important because there are many things that can affect the LC50, and unless this information is reported, no one else can use the data. Under information about the fish, one should report both the scientific and common names, the age, life stage, sex of the fish, and the range of the lengths or weights or both. Very often people report where the fish were obtained and their condition, any treatments used on them, and the holding and acclimatization procedures used.

B About the Physical Setup

Information on the physical setup should include the type of test chambers used, the volume of water used, the number of fish per test chamber, and the average grams of fish per liter of water and experimental design. For continuous-flow tests one should report the flow rate.

C About the Toxicant

Information about the toxicant should identify the source of the toxicant and its composition. One should also describe the formulation of stock solutions used to introduce the toxicant into the test chambers.

D About the Water

Information about the water should include the pH, alkalinity, dissolved oxygen, hardness, total dissolved solids, and temperature. Conductance and acidity may be useful. Calcium, magnesium, sodium, potassium, chloride, and sulfate measurements can help characterize the water. Many people also report the source of the water and any pretreatment, such as aeration, activated charcoal, or softening. One should also measure and report any unusual constituents in the water, constituents present in unusual amounts, or constituents which are known to affect markedly the toxicity of the material under test.

Once you have decided what measurements to make, it is important to use a good method for the determination. One is actually better off having no information rather than having wrong information. I would recommend use of methods from the EPA manual titled "Methods for Chemical Analysis of Water and Wastes" whenever possible. The manual has two basic purposes:

1. To identify the simplest possible legally defensible methods;

2. To promote standardization so that results will be comparable from one laboratory to another.

III OTHER TOXICITY TESTS

The same kinds of information should be reported for any other kind of toxicity test with any other test organism.

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Descriptors: Bioassay, laboratory tests

THE STATISTICS OF TOXICITY TESTS

I EXPERIMENTAL DESIGN

In general terms a toxicity test is used to investigate the effect of a toxic agent on a test organism. Because of biological variation between individuals, in order for the test to be meaningful, the effect must be studied for a group of individual test organisms, not just one individual. In addition, an investigator generally runs a toxicity test to determine the level of a toxic agent that will produce a given degree of effect, i.e., to determine an endpoint. Therefore, in practice, a toxicity test is usually run to determine the level of a toxic agent that produces a defined endpoint in a population of test organisms. Usually this is accomplished by exposing portions of the population to different levels of the toxic agent and observing the effect of the toxic agent on the various portions. In the Acute Mortality Test, this means observing the percent that is killed at each level of the toxic agent and then determining the level that would kill 50 percent of the population. For a toxicant, this is called the median lethal concentration (LC50). This test procedure imposes certain requirements on the experimental design of the test if the results are to valid.

A. Randomization

The first requirement is randomization, both of the test animals and the test chambers. Randomization of the test animals is important so that the portion of the population exposed to each level of the toxic agent is representative of the whole population--at least as representative as one can make it. Randomization of the test chambers is important to minimize the effects of external factors on the results of the test. There are several ways randomization can be performed, such as by drawing cards out of a hat or using a table of random numbers. In general, stratified randomization is better than total randomization.

B Replication

The second requirement for a good toxicity test is replication of test chambers. Duplication is about as far as most investigators will go. Replication is needed because randomization cannot overcome all problems. One must get an idea how much variation exists in the test, and the only way to do this is through replication. There are two kinds of replicates--those run at the same time and those run at different time. Sometimes it is said that replicates run at the same time measure reproducibility, and those run at different times measure repeatibility.

C Numbers of Subjects.

A third requirement is to have an adequate number of test animals in the population and in the portions of the population. This is replication of test animals. Five animals per portion is about a bare minimum; ten is a good compromise between theoretical and practical necessity. It is obvious that if only five animals are used in a portion, the results for a portion can only be 0, 20, 40, 60, 80, and 100 percent. A difference of one animal between duplicate portions means a difference of 20 percent. If there are ten animals per portion, a difference of one animal will mean only a 10 percent difference. Even the best random distribution cannot make the replicates identical, so there must be enough animals in each replicate to minimize the consequences of such differences.

D Number of Partial Kills

(1) A fourth requirement of a good experimental design for toxicity tests is that there be enough levels of the toxic agent tested so that the level producing 50 percent kill can be determined accurately. If one only tests levels that kill either 0 percent or 100 percent of the animals, all he knows is that the LC50 is between two of the levels tested.

If, on the other hand, the investigator tests levels that kill 0, 20, 40, 60, 80, and 100 percent of the animals, he can determine the LC50 rather accurately. There are biological and practical limitations on how accurately one can determine an LC50. However, if results are to be very meaningful, as a bare minimum the test should have two concentrations that produce partial kills. With two partial kills, one can calculate the LC50 and its confidence limits with fair accuracy.

(2) The degree to which an investigator worries about each of these requirements must depend on the confidence one wants to have in the data. One can ignore all four requirements and still get a "ball park" figure, but "ball park" results are not very useful. Of course, it is ridiculous to go into great detail on the statistical requirements for a good toxicity test and ignore other things such as the biological and chemical requirements.

II ANALYSIS OF THE DATA

A Once the test has been run, the remaining problem is to calculate the results from the data collected. However, what can be done with the data is often limited by what data were collected and how they were collected. Thus the design of the experiment should take into account the ultimate use planned for the data. The only data collected from most routine toxicity tests is concentration-mortality data.

B There are several methods for analyzing concentration-mortality data. It is generally accepted that the best way is to determine the median lethal concentration (LC50). Statistically this is a good endpoint and it is about as useful as any other one. All of the methods for calculating an LC50 can be visualized as graphical methods based on a plot of concentration vs. percent mortality. What changes from method to method are the coordinates and the means of connecting the points. Standard Methods describes the commonest graphical method, but in general graphical methods are approximate methods, give no measure of confidence limits, and are not useful with certain kinds of data. Most people who want to use a better method use the Litchfield-Wilcoxon method. This is a semi-graphical approximate probit method, but this is rather time consuming unless a computer can be used. Others use various other parametric or nonparametric methods, such as the logistic method of Bergson, or a moving average method. All of these are discussed by Finney. Some of these methods assume a particular relationship between concentration and mortality and some do not. Generally it is impractical to try to get enough data to prove whether or not a specific relationship exists. Fortunately, most often the calculated LC50 and its confidence limits are about the same for all computational methods.

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Descriptors:

Bioassay, Statistical tests, Laboratory tests

THE SELECTION OF ENDPOINTS FOR TOXICITY TESTS

I INTRODUCTION

An endpoint in a toxicity test is a defined magnitude of a specific observed effect of a toxic agent on a living organism. Thus the selection of an endpoint involves both the selection of the effect and the selection of the magnitude. At one time "toxic" was generally used to mean "to cause death" and death was almost the only effect studied. However, today it is generally recognized that toxic agents cause many damaging effects other than death, and so "toxic" is used to mean "to cause an adverse effect" and "lethal" is used to mean "to cause death."

II DEATH

A Death was probably the first effect used in toxicity tests and is still the most widely used effect because it possesses four very useful properties:

(1) It applies equally well to all organisms;

(2) It applies equally well to all toxic agents;

(3) Usually it can be detected rather easily without the use of specialized equipment;

(4) It is an obviously important adverse effect.

Because of these properties, death will probably always be the basic observed effect for toxicological studies.

B If an endpoint is to be defined using death as the effect, in terms of a group of subjects, there are several magnitudes of death that can be used, such as:

(1) the lowest concentration that kills all of the subjects (LC100)

(2) the concentration that kills 50% of the subjects (LC50)

(3) the highest concentration that kills none of the subjects (LC0).

C It has been found that statistically and practically the LC50 is the best endpoint. Although it is sometimes argued that LC0 should be a more useful endpoint, the LC0 is difficult to determine.

III OTHER EFFECTS

There are many effects other than death that can be and have been used. Warner (1967) reviewed many histological, physiological, biochemical, behavioral, activity and growth effects and endpoints. The possibilities are only limited by man's ingenuity, time, and money.

IV CRITERIA FOR THE SELECTION OF ENDPOINTS

A From all the effects that can possibly be used for toxicity tests, one must choose the best effect for one's own tests. Death is obviously widely used effect, but it is generally not sensitive enough. From the more sensitive effects one must choose one that is practical and will meet the needs of the experiment.

B Generally the first consideration is time and equipment, and these are obviously important, but usefulness should be the primary concern. Water pollution control is a matter of solving practical problems. Thus an effect should be useful. For many of the effect that can be used in short, sensitive tests, there is no information on usefulness and some of these require elaborate equipment.

V CHRONIC TESTS

Much of the work of the National Water Quality Laboratory is now centered around what we call the chronic test. In these tests the animals are exposed to the toxic agent before and during spawning and the eggs and fry produced are exposed for about thirty days or more. In these tests, we look for effects on survival, growth and reproduction, because such effects are obviously important and are rather easy to study. Chronic tests generally last for eleven months or more. In addition, we are studying the usefulness of some other sublethal effects by comparing the results of acute tests with those of the chronic tests.

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Descriptors: Bioassay, Methodology, Laboratory Tests

I INTRODUCTION

A The report of the Council on Environmental Quality (1970) repeatedly stresses the need for the development of predictive, simulative, and managerial capabilities to combat air and water pollution. The last capability depends on the first two.

B The standard static jar fish bioassay, which uses death as a response, enables one to predict the toxicity of a particular waste to fish. One limitation of this procedure is that it uses a grab sample which represents the quality of the waste at only one point in time. The water used to make the dilutions is also taken at one point in time. At the actual industrial site, the quality of the waste and the river water vary through time. A composite waste sample partially overcomes this limitation, but may mask variations that are biologically important.

C One could put fish in a continuous flow of waste diluted with river water, but then there is one further limitation of the standard bioassay: death is used as the response. In order to prevent damage to organisms, it is necessary to have an early warning of dangerous conditions, so that corrective action can be taken. In other words, symptoms of ill health, which occur before death, must be detected if there is to be time for diagnosis and treatment.

II METHODS AND MATERIALS

A Fish Movement Patterns

1 Fish movement patterns can be monitored using the technique of light beam interruption described in detail by Cairns, et al. (1970). Dawn and dusk are simulated by a motor-driven dimming unit which gradually increases the intensity of the room lights over a half-hour period starting at 6:30 a.m. and gradually decreases the intensity to 0 over a half-hour period starting at 6:30 p.m. The cumulative movement of each of six bluegill sunfish, a single fish per tank, is recorded every hour throughout a test except during the simulated sunrise and sunset when an additional record is made on the half hour. Each day is divided into four intervals; first half day, second half day, first half night and second half night (Table I). Before any statistical analysis can be performed, recordings for day 1 must be completed. After the cumulative movement for day 1 is recorded, statistical analyses are performed after the completion of each designated time interval. For example, the cumulative movement recorded hourly for each fish during day 1, first half day values are compared to the cumulative movement recorded hourly for each fish during day 2, first half day values.

2 Based on the results of 20 laboratory experiments "stress detection" is defined as the presence of two or more abnormal movement patterns recorded during the same time interval.

B Fish Breathing

1 Breathing rates may be determined from polygraph recordings of breathing signals. The fish are tested in plexiglas tubes through which dechlorinated tap water or some toxic solution is metered at a flow rate of approximately 100 ml/min. Breathing signals are detected by three platinum wire electrodes placed in the water; an active electrode, an indifferent electrode, and a ground. The test chambers and methods of acclimating the fish are described in more detail by Cairns, et al. (1970) The photoperiod is the same as that for the fish movement study.

2 The fish are placed in test chambers by 6:00 p.m. and the recordings began at 6:00 a.m. the next day to allow the fish to recover overnight from handling. Toxic solutions are introduced at 10:00 a.m. after the experimental fish have been exposed to water containing no added

toxicant for periods of one to six days. Each experimental fish thus serves as its own control. In addition, one or two fish are never exposed to the toxicant and serve as controls throughout each experiment. In one experiment, using zinc as the toxicant, reported in Table VI, six control fish were exposed to water containing no added zinc for four days.

a Preliminary exidence suggested that the data could be analyzed by separating the experimental day into four periods; a period from 6:00 to 8:00 a.m. when the breathing rates changed markedly, a period from 9:00 a.m. to 5:00 p.m. when the rates were comparatively high, another period of rapid change from 6:00 to 8:00 p.m., and a night period from 9:00 p.m. to 5:00 a.m. when the rates were comparatively low (Sparks, et al., 1970).

b Bluegills increase their breathing rates when exposed to zinc (Cairns, et al., 1970). An individual fish was thus considered to have shown a response each time its breathing rate during a time period exceeded the maximum breathing rate observed during the corresponding period of the first day, before any zinc was added. A response was scored for each value on the second day that was higher than the first day maximum for the comparable period. The control periods (before any zinc was added) and the experiment where no zinc was added at all were used to determine how many false detections this method of analysis would produce. The experimental periods (after zinc was added) determined how quickly the method of analysis could detect zinc concentrations in water.

c Zinc concentrations were determined daily by atomic absorption spectrophotometry.

III RESULTS

A Fish Movement Patterns

1 Table 1 shows the results of one continuous flow experiment carried out for 20 days. During this experiment fish were exposed to zinc on day 7 from 1:00 p.m. until 7:00 p.m. at which time the flow was returned to normal dilution water. The zinc concentrations reached their maximum at 7:00 p.m. and atomic absorption analyses on effluent samples collected at this time showed the following concentrations: tank one, 13.32; tank two, less than 0.08; tank three, 11.39; tank four, 12.72; tank five, 13.32; and tank six, 12.59 mg/l Zn^{++}. The results show that these concentrations of zinc developing over the six hour interval of exposure were insufficient to cause a detectable change in the movement patterns of the fish. By 8:30 a.m. of day 8 the effluent zinc concentrations were less than 0.30 in all cases.

2 To determine the percent survival and recovery patterns of the fish once stress detection occurred, zinc flow was reinitiated at 1:00 p.m. on day 13 of this experiment. Between 8:00 and 9:00 p.m. on day 13 the zinc concentration in the effluent reached a maximum of: 7.51 for tank one; less than 0.05 for tank two; 7.49 for tank three; 7.52 for tank four; 7.49 for tank five; and 7.54 mg/l for tank six. The concentrations remained near the above values until the statistical analyses showed "stress detection" during the first half night values on day 14 (Table 1). As soon as stress detection occurred the flow was returned to normal dilution water. At 10:00 a.m. on day 15 zinc analyses showed all effluent concentrations to be less than 0.70 mg/l Zn^{++}. Stress detection continued to be registered for two consecutive time intervals following the initial detection, but after that no stress detection was registered and the frequency of abnormal patterns returned to prestress levels within 48 hours. In this experiment

as with all others in which dilution water containing zinc was replaced with dilution water minus zinc at that time of stress detection all fish survived!

3 The results from the series of experiments at progressively lower zinc concentrations indicate that the lowest detectable concentration is between 3.65 (Table II) and 2.93 mg/l zinc (Table III) for a 96-hour exposure.

B Fish Breathing

1 Table IV shows the breathing rates of five fish on days 1, 2, and 7 of experiment 8. The first four fish were exposed to a measured zinc concentration of 4.16 mg/l, beginning at 10 a.m. on day 7. The fifth fish served as a control and was not exposed to any added zinc. The amplitude of the breathing signals decreased every night, and the breathing rates for fish 2, in particular, could not be determined during some portions of the dark period (7:30 p.m. - 7:00 a.m.). The maximum breathing rates for each fish during each period of the first day are circled. The breathing rate of any fish during a time period of day 2 or day 7, which is greater than the maximum breathing rate recorded for that fish during the corresponding time period of the first day has a rectangle drawn around it. The total number of fish showing increased breathing is given at the bottom of each column. On day 2, fish 2 showed increased breathing on just two occasions. In contrast after zinc was added on day 7, three and four experimental fish at a time showed increased breathing.

2 Table V summarizes the results of successive comparisons of the first day maximal breathing rates to breathing rates on subsequent days (SCM method of analysis), for experiment 8. During the control period before any zinc was added there were 15 occasions when a single experimental fish responded, and three occasions when two experimental fish responded at the same time. At no time during the control period did more than two fish show responses together.

After the zinc was introduced, all four of the exposed fish showed responses simultaneously on five occasions, and three fish showed responses during the same time interval on 19 occasions. If the criterion for detection of water conditions potentially harmful to fish were two or more responses during the same time period, then three false detections would have occurred before any zinc was added, and 4.16 mg/l zinc would have been correctly detected eight hours after it was introduced. If the detection criterion were three or more responses during the same time period, then no false detections would have occurred and the zinc would still have been correctly detected after eight hours.

3 The lowest zinc concentration tested was 2.55 mg/l. Using a detection criterion of simultaneous responses by three fish, this concentration was detected 52 hours after the zinc was added, with no false detections occurring during the four hours before zinc was added (Table VI). The responses of six control fish that were exposed to dilution water containing no added zinc are also shown for comparison. Note that there was no tendency toward increased breathing rates through time in the control fish, and that no more than one control fish showed an increased breathing rate during one time period.

4 Table VII summarizes information on three experiments that indicates the effectiveness of the SCM method of analysis when different criteria for detection are used. Changing the criterion for detection from one to three responses per time period generally increases the lag time and decreases the number of false detections. The lag time is the time from the addition of zinc to the first detection. A false detection is one occurring before any zinc is added to the water.

IV DISCUSSION

A The experiments described above show that the movements and breathing rates of bluegill sunfish can be used to detect sublethal concentrations of zinc. The criterion for detection is a certain number of fish showing an arbitrarily defined response in breathing rate or activity during one time period.

B In choosing a specific criterion for detection, the risk of not detecting stressful conditions soon enough must be weighed against the risk of false detections, and the choice would probably be determined by the nature of the pollutant. If a pollutant is easily detected by the biological monitoring system, is slow-acting, and if the toxic effects are reversible, then the criterion for detection might be responses by 3/4 of the test fish, to avoid the false detections that would necessitate expensive remedial action or a temporary shut-down. On the other hand, an industry that produces an effluent containing a fast-acting toxicant whose effects are irreversible would probably use a criterion that leads to rapid detection (responses by 1/4 to 1/2 of the test fish), and would have to go to the expense of installing holding ponds or recycling facilities to accommodate a relatively high number of false detections. Alternatively, a safety factor could be introduced by metering proportionally more waste into the dilution water delivered to the test fish than is delivered to the stream. The safety factor could be determined by growth and reproduction experiments with fish.

C In an actual industrial situation water and waste qualities are apt to vary unpredictably, and it would certainly be desirable to have a redundant detection system. It is conceivable that some harmful combination of environmental conditions and waste quality would be detected by monitoring one biological function, but not by monitoring another. It is also possible that excessive turbidity would disrupt the light beams of the movement monitor, and not affect the breathing monitor; or that an excessive concentration of electrolytes would affect the electrodes of the breathing monitor, but not affect the activity monitor. Therefore, the activity monitor and the breathing monitor have been combined in our laboratory for further experiments (Fig. 1).

D The rate of data acquisition and analysis could be greatly speeded up if the monitoring system were automated as shown in Figure 2. The sampling rate would be controlled by a minicomputer which could receive data from the movement monitor and the polygraph via a multiplexer as often as every minute. The minicomputer would be programmed to perform statistical analyses every 10 minutes, for example, and output the results on a teleprinter.

E Figure 3 shows how the fish monitoring units would be used at an actual industrial site. A monitoring unit would be located on each waste stream in the plant and on the combined waste stream. The experimental fish in each unit would be exposed to waste diluted with water from the river above the plant, and control fish would be exposed to upstream water alone (Fig. 4). The information from each monitoring unit could be analyzed by a central data processor, and when there was a warning response, the industry could tell which waste stream was at fault. If the problem was outside the plant, the control fish would show responses.

F Figure 5 shows how the in-plant monitoring systems would be integrated into a river management system. The in-plant monitoring units are shown as squares, and in addition to supplying information to each industry, the monitoring units also inform the control center. In such a system, there are several alternative damage prevention measures that could be used, in addition to whatever measures, such as shunting wastes to a holding pond or recycling wastes for further treatment, are available to each industry. If the monitoring units at Industry 2 indicate that toxic waste conditions are developing, then the control center might have Industry 1 hold its waste until the danger of combining wastes from Industry 1 and 2 in the river were alleviated by control measures at Industry 2. Alternatively, the control center might call for a release of water from the upstream dam to dilute the effluent from industry.

G It is likely that "fish sensors" in continuous monitoring units at industrial sites can warn of developing toxic conditions in time to forestall acute damage to the fish populations in streams. In conjunction with stream water quality standards for chronic exposure, such biological monitoring systems should make it possible for healthy fish populations to co-exist with industrial water use.

ACKNOWLEDGEMENT

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Descriptors:
Environmental Control, Bioassay, Toxicity, Fish Behavior, Fish, Monitoring

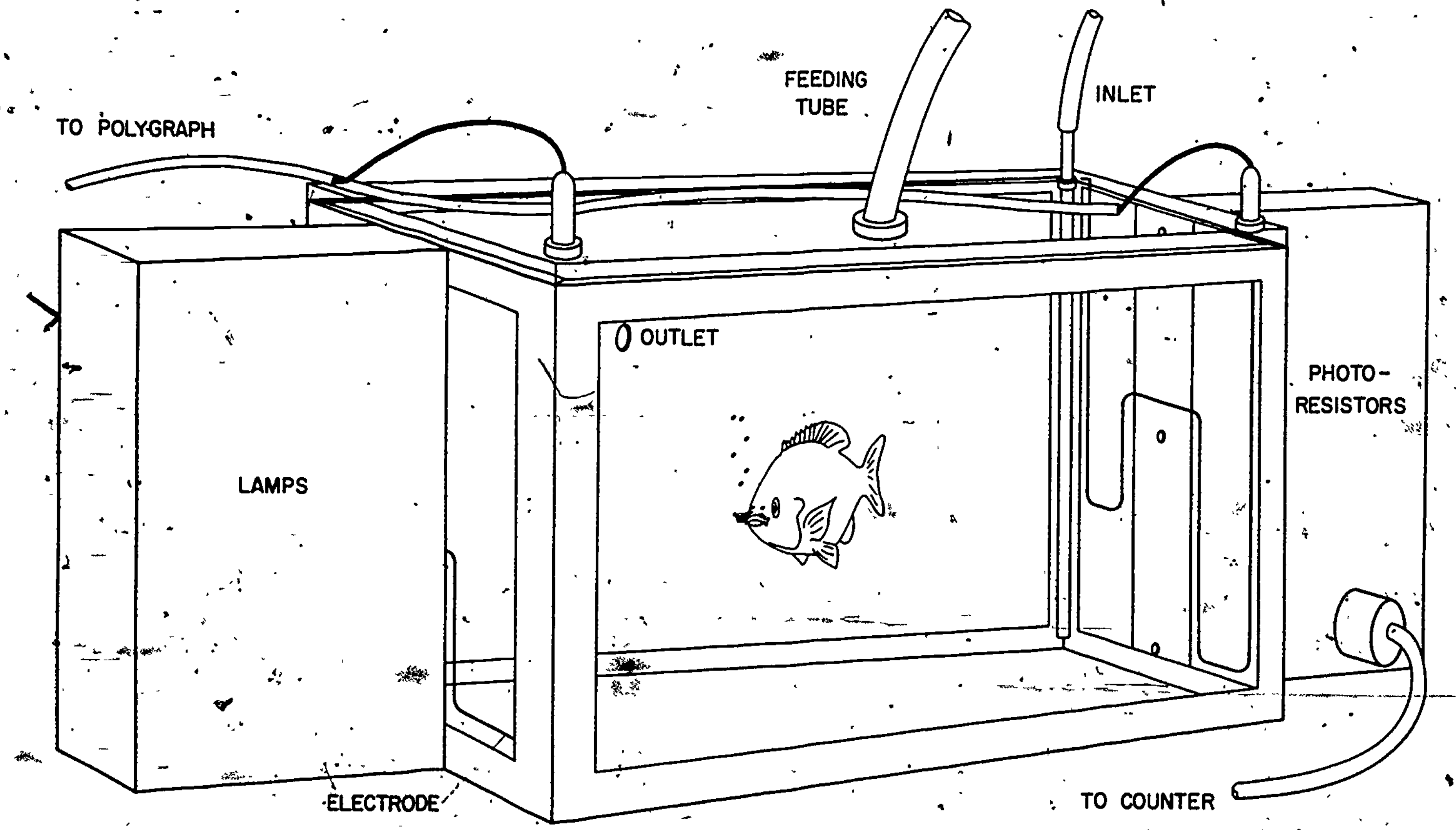

Figure 1. Test chamber for monitoring system, showing the electrodes for recording fish breathing and the light-beam system for recording fish movement.

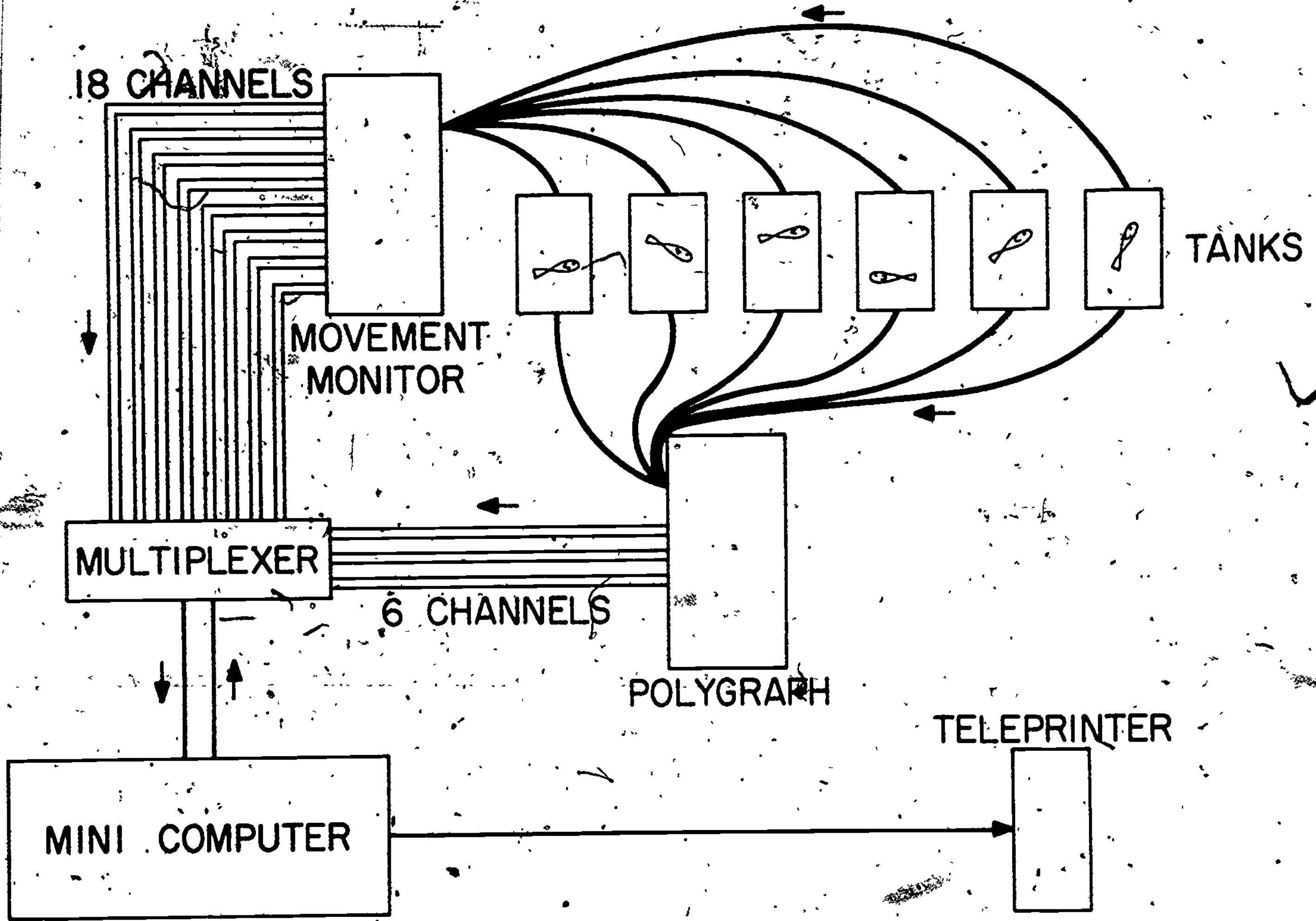

Figure 2. An automated fish monitoring unit.

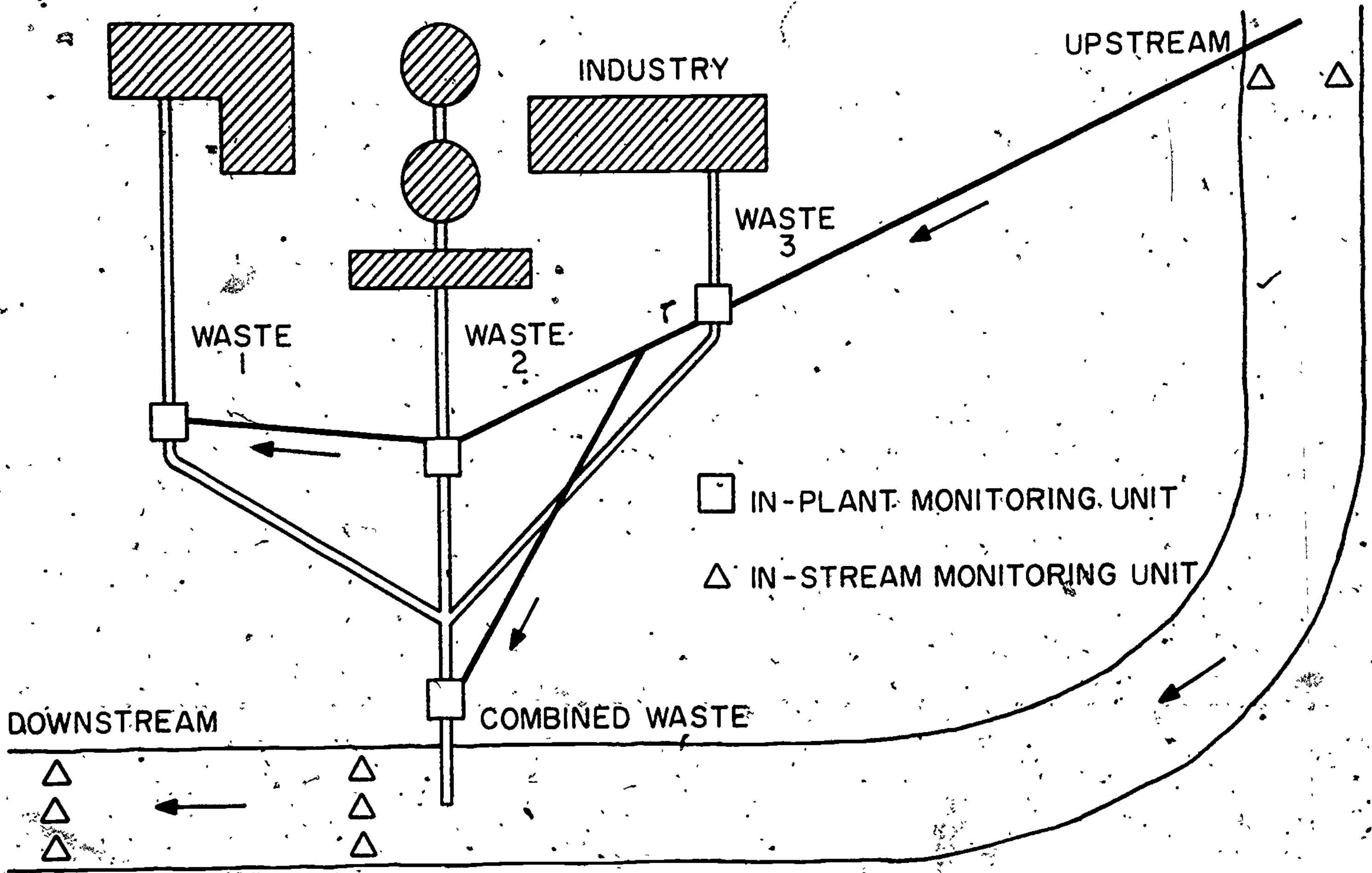

Figure 3. Arrangement of fish monitoring units at an industrial site.

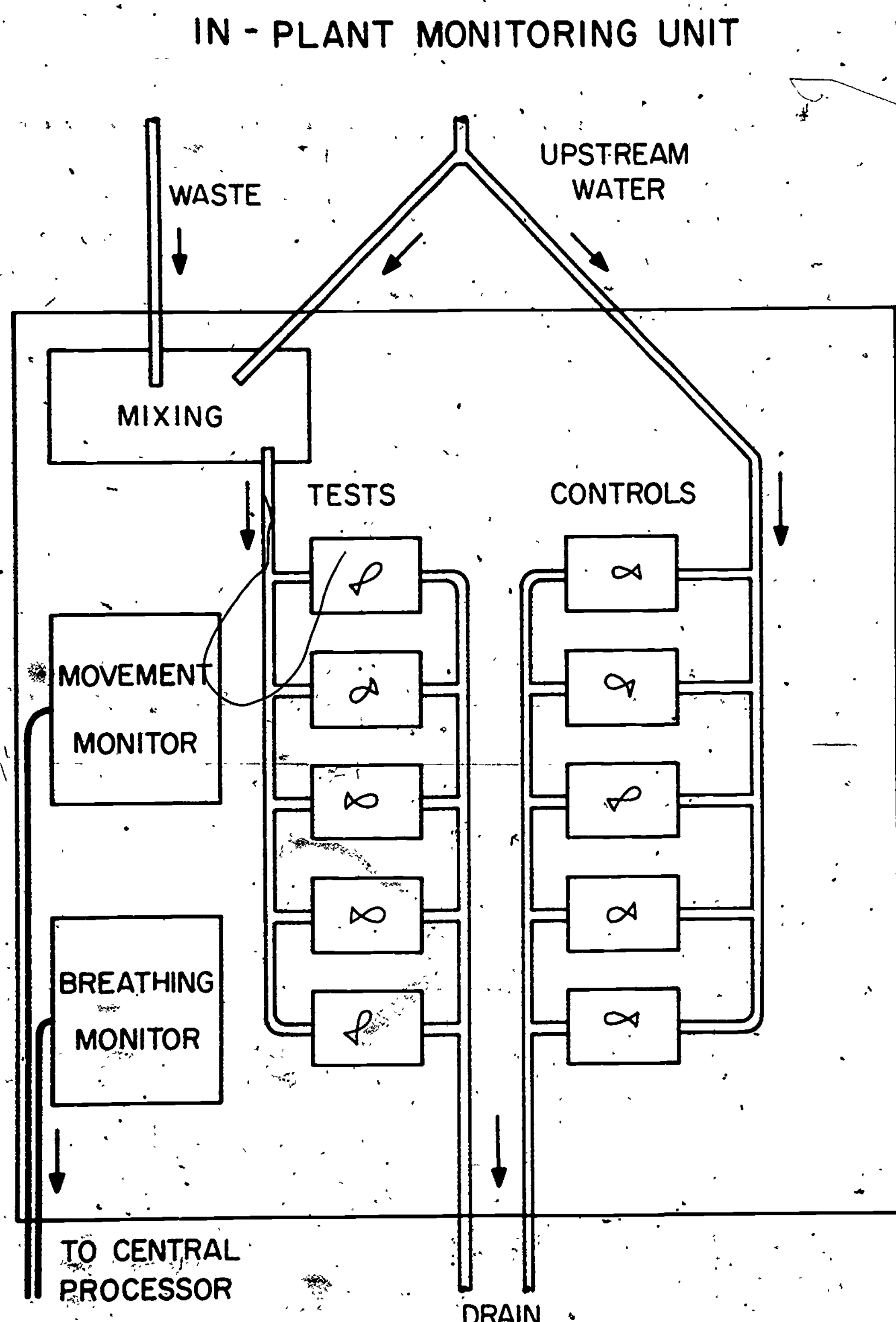

Figure 4. Detail of a single fish monitoring unit, showing how the experimental fish are exposed to waste diluted with upstream water and the control fish are exposed to upstream water alone.

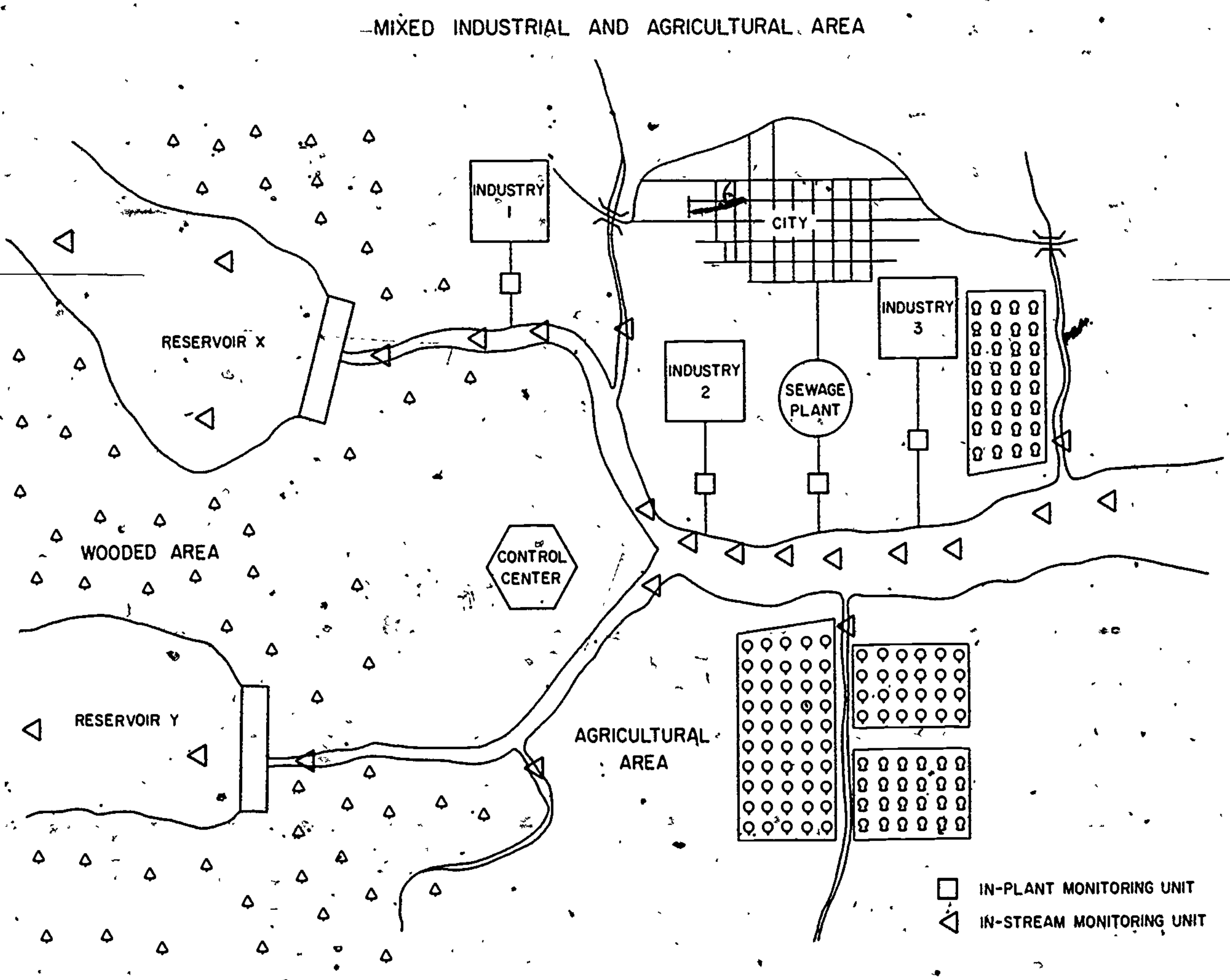

Figure 5. Use of in-plant monitoring units in a river management system.

ECOLOGICAL CONSIDERATION IN PLANNING WATER QUALITY SURVEYS

I INTRODUCTION

A Ecologists must be willing to take a chance and predict the ecological consequences of alternative schemes for water resources management if we are to realize the maximum beneficial use of our resources. Historically, ecologists have lagged behind their engineering colleagues in developing prediction capabilities for a number of reasons.

1 Unfortunately, the operational characteristics of ecosystems are poorly understood when compared to the engineers systems. Engineers can predict that with so much concrete, steel, etc., and with a given amount of labor and money, a dam can be built on a river which will enable them to regulate flow behind the dam. The flow figures can be predicted with reasonable accuracy, and when the dam is built, the performance is generally within the original estimate. However, due to the complex chemical, physical and biotic interactions of an ecosystem prediction of the ecological consequences of any activity is more involved. The ecologist has developed only relatively recently rather primitive prediction systems for complex natural environments.

2 Another reason that ecologists have lagged behind engineers is that appropriate channels of information exchange between them have not always been open. Two contrasting philosophies have existed in the past which have hindered the ecological management of our water resources. Engineers, water resource economists and industrialists have generally had a construction philosophy of life which has conflicted with the conservationists or protective philosophy. Those who build power plants, dams, reservoirs, canals, etc., have used the technology of the time most economically appropriate with the expectation that what they are building will have a very short life span in terms of geologic time, and that when the structure is outmoded or uneconomical, it will be torn down or replaced. Plans are also strongly time dependent, and once completed must be initiated in a relatively short time period or they will become outdated. Complex sets of conditions involving technology, financing, land acquisition, power demands, etc., prevail; thus, the engineer is often characterized by a time and tide wait for no man attitude. The conservationist, on the other hand, realizes that once a rare species endemic to any area is lost, it is gone for all time, and that ecosystems once damaged may be difficult, if not impossible, to restore to their original condition.

B Fortunately, a new awareness on the parts of the advocates of both philosophies that our life support system on earth has two components, one industrial and the other ecological, has forced ecologists and engineers to work together. We currently realize that the survival of our present social system depends upon our ability to develop a harmonious relationship between these components.

C Those people involved in water resources management realize that some of the frustration and public outcry about water resources projects could have been avoided if proper consideration was given to ecological information before construction. Present trends in legislative action combined with the ever growing concern over the quality of the environment, the "environmental impact" of any new water resources development will be closely scrutinized. It is our objective here to briefly present some ecological information that should be considered along with other parameters in river basin planning.

II PRECONSTRUCTION ECOLOGICAL SURVEY

A One of the common problems of water resources development is to select a project site which allows maximum use without environmental degradation. In order to get this type of information, it is necessary to develop a series of prediction systems which will allow an ecologist to rank the potential construction sites. Perhaps one of the best ways to obtain important types of ecological information to be used in river basin planning is through a preconstruction ecological survey.

1 A preconstruction survey should be carried out by a team of chemists, ecologist, engineers, and taxonomists to get a complete picture of the chemical, physical and biological condition above and below the potential site location. If adequate background data are to be generated, the team should consist of one or more chemists, a bacteriologist, an algologist, a protozoologist, one or more invertebrate zoologists (including an aquatic entomologist), and ichthyologist, and a sanitary engineer. Since this involves a number of well-trained people, it can be moderately expensive.

2 The exact cost would depend on a number of factors including the size and structure of the river and the number of species likely to be encountered. Obviously, the lower Mississippi is a more difficult river to survey than a small river that one can throw a rock across. In addition, a stream already degraded by pollution is likely to have fewer species resulting in less cost for identifying the various organisms collected than an unpolluted stream with a very high number of species.

3 Before such a survey is contemplated, it is well to have a preliminary survey by a generalist used to dealing with these problems who can make a firm estimate of the costs involved and place reliable time estimates on completion of the project.

B A survey of this nature will provide a wide variety of information valuable in making a choice between prospective project locations.

1 It will establish a baseline of biological, chemical and physical water quality which can be useful in determining the waste assimilative capacity and other beneficial uses of the system. If one views the waste assimilative capacity of a river as a natural resource, then it is only logical to make use of that capacity along with other uses such as water supply, recreation and aesthetics to derive the maximum beneficial use from the system.

2 A preconstruction survey will determine pre-existing man made or natural stresses on the receiving system. In order to avoid blame, there is no better defense than an aggressive offense. Preconstruction data which documents the water quality is extremely valuable, particularly in receiving systems which are already partially under stress from other waste discharges. It is essential to establish the presence of natural stress on a system and thus avoid blame after project construction is completed and operations begin. Natural stress can take range from siltation and the introduction of organics from leaf litter, to thermal changes due to the introduction of underground aquifers.

3 How many water resources projects do you know that are located near critical spawning areas of striped bass, just above, or in the middle of an important fishery, in an area where the aquatic life is particularly vulnerable, and the like? Many of these situations could have been avoided through a preconstruction survey before site selection was made. Alterations in design of discharges could have received valuable input information based on this identification of valuable wildlife resources. For example, in some cases, it might be desirable to design waste discharge systems so that the waste is held against one bank of the stream or river leaving a free channel on the other side where migratory fish could pass through the area.

4 A preconstruction survey is a convincing demonstration that the resource developers are sincere in their efforts to protect the environment. The information derived from the survey can often furnish information about the ecological history of the area and make some predictions about future trends,

all of which are useful to engineers designing the waste disposal system and to the administrator concerned about public relations.

5 Through identification of critical physical, chemical and biological parameters, the preconstruction survey can help water quality personnel predict the mixing zone upon project operations. This is an important factor from a regulatory as well as public relations viewpoint.

III PREDICTIVE BIOASSAY

A Of equal importance to the preconstruction survey in project site selection is the availability of toxicity information based on a predictive bioassay. Even if a building site for an industry has been selected, but before construction begins or preferably before final designs of the plant are approved, some bioassays should be carried out.

1 These should be carried out with a simulated plant waste as close as possible in quality to the anticipated operating waste under the worst possible conditions.

2 Ideally, bioassays should contain three elements of the food chain, i.e., primary producers such as algae, invertebrates, and a fish. Just because a waste doesn't kill fish directly, it may ultimately prevent their survival due to its toxicity to an intermediate in the food chain. It is important to protect other elements of the aquatic community besides fish. For example, the loss of algae may impair the ability of the aquatic system to receive and transform organic wastes.

3. When conducting predictive bioassays, water quality of the proposed site location should be used since there are documented examples of synergistic interactions resulting in increased toxicity. If a plant is considering locating a system already receiving heavy metal discharges and plans to add additional heavy metals, then a predictive bioassay using the receiving water quality is essential since, for example, the toxicity of zinc combined with copper is ten times their individual toxicity.

B Predictive bioassays can be useful in helping determine a site selection for a variety of reasons. For example, an industry having a discharge containing heavy metals should consider locating in an area where the hardness of the water is high since for zinc the 96 hour TL50 in hard water is 10.1 - 12.5 ppm for the bluegill sunfish, where the 96 hour TL50 in soft water is 2.9 - 3.8 (McKee and Wolf, 1963).

C Predictive bioassays can be used to allow the plant to make maximum use of the system, to identify potentially hazardous interactions, and to see if preliminary waste treatment design is likely to be adequate, and if not, can be used to estimate the degree of additional treatment required.

D No longer can an industry be solely concerned with determining the acute toxicity of its waste products. Bioassays of industrial wastes have progressed from short-term tests using a single species with death as an end point to long-term tests involving several species and even communities of organisms. The use of respiration, growth, reproductive success, electrocardiogram movement patterns or other functional changes may replace the use of death as a criterion of response. The latter requires more time and expense but provides valuable predictive information concerning the "biologically safe concentrations" of various toxicants.

IV SIMULATION TECHNIQUES

A The development of simulation techniques involving the use of scale models becomes increasingly important as our population grows and more intensive use is made of the finite space available to us. In the past when we damaged an environment seriously, we could move on to a new undamaged environment and avoid most of the immediate consequences of poor management. Perhaps the last big movement of this sort in the United States was the exodus from the Dust Bowl. However, since most of the ecosystems of the United States are at or near tolerable stress levels, we no longer can go to virgin territory and escape our environmental mistakes. As a consequence, we can afford fewer mistakes without immediate penalty than we could in the past.

B One of the obvious protective measures we might take to prevent major ecological or environmental problems is to simulate prospective new uses in scale or laboratory models and restrict most of our mistakes to these. This practice is too common in engineering (for example, the U. S. Army Corps of Engineers river models at the Waterways Experiment Station, Vicksburg, Miss.) and industrial circles that it would hardly need mention were it not for the fact that ecological scale models or environmental simulation systems are not now commonly used.

C However, ecologists now are becoming quite interested in developing scale models to simulate various environmental systems and the practice should become increasingly common in the future. Of course, these suffer the weaknesses of all scale models and are still in primitive stages of development. They need not be extremely expensive and may be used to generate data which could be useful in preventing large scale mistakes. For example, many of the events which have occurred in Lake Erie could probably have been simulated in models.

D An example of a scale model we commonly use in our laboratory is a model stream to which we have attached a model steam condensing system allowing an incremental increase with a variable contact time in the condensor (Figure 1). Water from the model stream is passed through the condensor system and then through a series of plexiglass troughs where we allow algal and protozoan communities to establish. These experimental troughs are compared to control troughs, and some predictive information on the effects of passage through the system on downstream community structure determined.

V ECOLOGICAL QUALITY CONTROL TECHNIQUES

A Since we are a society almost compulsively dedicated to change, we are desperately in need of adequate prediction systems. The preconstruction survey, predictive bioassay and scale models previously discussed allow the ecologist ot make some of these predictions and help identify the various alternative uses which might be made of the environment and to estimate what the consequences of these will be. If these techniques were utilized in prospective water resource projects, we would be on our way to having adequate environmental planning. However, environmental planning alone will not be effective unless good quality control techniques are developed, as well as adequate environmental management practices.

B Just as in an industrial process where we have a system of checks and balances to insure product control, we must begin to develop the capabilities for environmental quality control provided an equitable environmental use plan can be developed.

1 This will require rapid biological, physical and chemical information systems, so that we get a continuous flow of information enabling us to predict unfavorable changes in our water resource systems.

2 Of the three types of information systems previously mentioned, the development of rapid biological monitors providing continuous information has lagged behind the other two in development. We can continuously monitor in a river many of the physical and chemical parameters

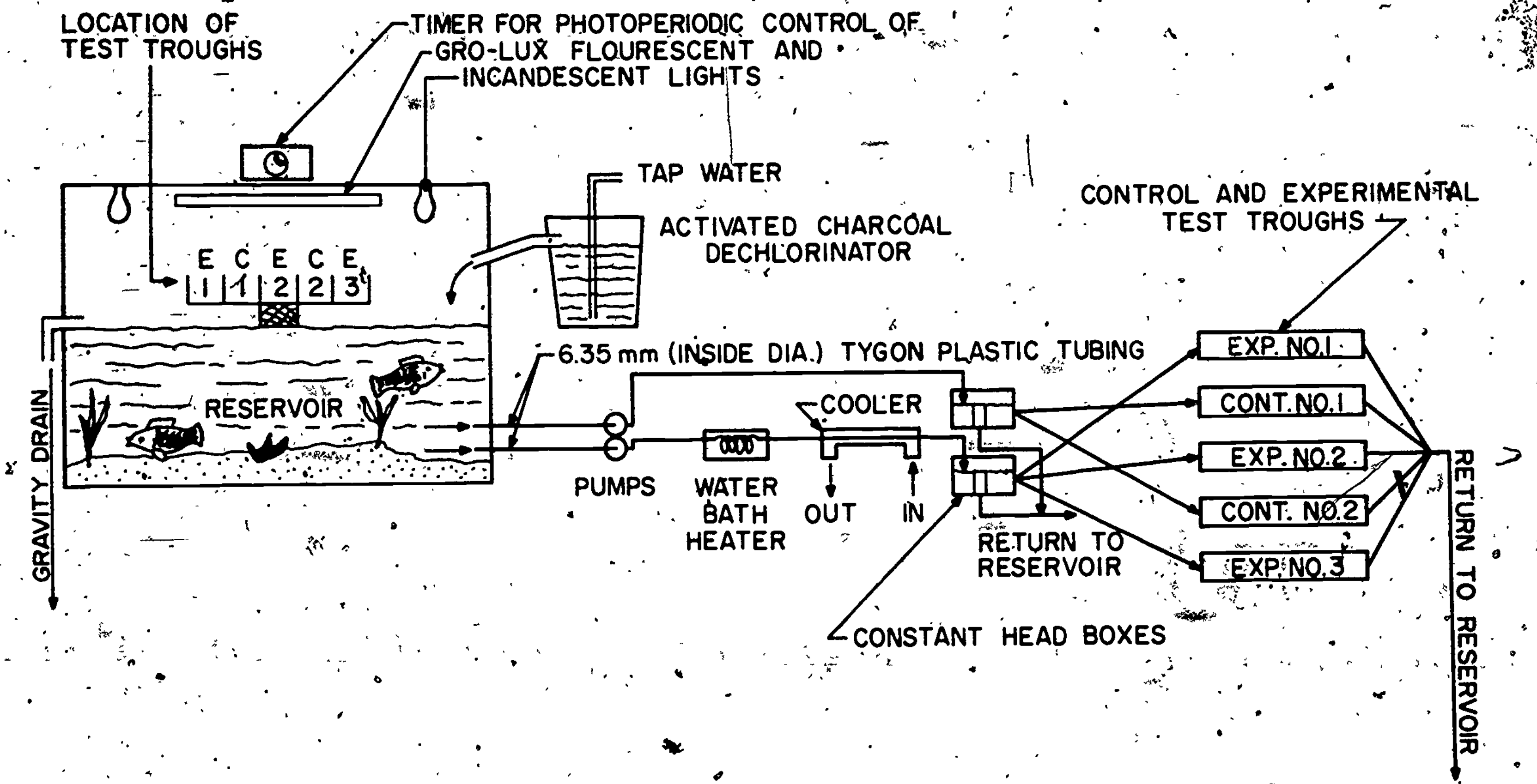

FIGURE 1.
SCALE MODEL SYSTEM FOR SIMULATING THE EFFECTS OF PASSAGE THROUGH A STEAM CONDENSOR ON MICROORGANISMS.

for analyses. However, the development of rapid biological information systems, both in-stream and in-plant, is essential to the maintenance of adequate environmental quality control.

3 We need to know the effects on biological organisms of a waste discharge before it enters the receiving stream as well as the biological effects after it enters the stream, and this information should be produced rapidly. Present systems are much too slow in view of the fact that the constituents of a waste stream are likely to vary from hour to hour and from day to day. Potentially disastrous materials should be detected before they enter a receiving stream if at all possible and at the very least, before substantial damage has been done in the receiving stream itself.

4 Several potentially useful methods for rapid in-plant monitoring are being explored (Cairns, et al. 1969) and one rapid in-stream method is now operational (Cairns, and Dickson, 1971). The in-plant methods just mentioned use changes in heart rate, breathing signal, and movements of the entire fish within a container to detect sublethal concentrations of toxicants in a waste discharge. If successful, these and other "early warning" in-plant systems could be used to determine the toxicity of a waste before it left the plant so that the appearance of a harmful concentration of a toxicant would activate a control system and shunt the waste immediately to a holding pond or recycle it for additional treatment.

C This continual information about the toxicity of a waste should enable sanitary engineers to identify periods of operation likely to produce the most toxic wastes, as well as identifying those components of the production process which contribute most of the toxicity.

D Full development of useful early warning systems with rapid information feedback will probably take a number of years and will require the close cooperation of a variety of disciplines. No doubt, the early developmental period will have its share of failures, but it is highly probable that effective systems can be produced and that their use will substantially improve environmental quality control. Since the ultimate test of the effectiveness of a waste treatment process should be in the receiving stream, in-stream early warning systems also should be developed to insure a continual flow of information.

VI SYSTEMS MANAGEMENT OF WATER RESOURCES

A Present advances in biological monitoring combined with physical and chemical monitoring capabilities indicate that in the near future we can develop and operate a river basin with varied water resource uses to maximize beneficial use without ecological damage.

B Figure 2 illustrates a river basin management system which includes reservoirs, agricultural uses, industries and towns, etc. Conceptually, utilizing a central control center and rapid physical, chemical and biological monitoring systems ecological damage could be prevented, through the operation of the system as a whole rather than each water resource user being concerned only with his own discharge.

C If an industry in the system had a spill of toxic material which was rapidly detected through the continuous monitoring systems, the following activities might be coordinated by the control center:

1 Upstream reservoirs could increase discharges for dilution of toxicants.

2 Municipal water users could curtail use of water and depend on reserves until toxicity was dissipated.

3 Downstream industries could shunt to holding ponds to prevent synergistic interactions.

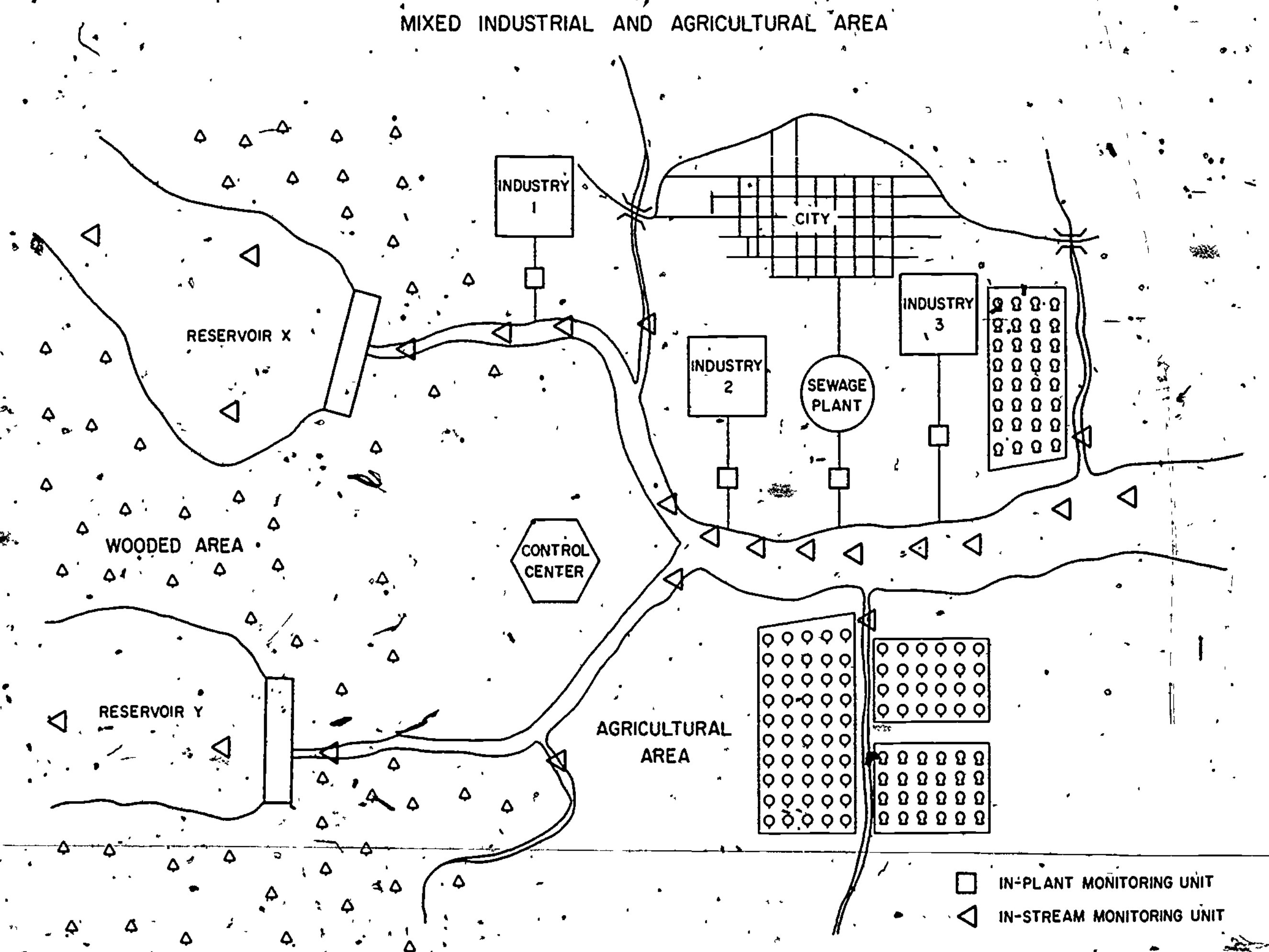

FIGURE 2
DIAGRAM OF A COORDINATED RIVER BASIN MANAGEMENT SCHEME

D Obviously, the water resources management scheme just outlined is optimistic and depends on the cooperative activities of state and Federal government as well as private users of water resources. However, we are rapidly approaching the time when technology is available to do this job. Implementation of such a program to protect and wisely utilize our water resources now depends on our sincerity.

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This outline was prepared by John Cairns, Jr., Research Professor of Biology and Director, Center for Environmental Studies, and Kenneth L. Dickson, Assistant Professor of Biology and Assistant Director, Center for Environmental Studies, Virginia Polytechnic Institute or State University, Blacksburg, Virginia 24061.

Descriptors:
River Basins, Surveys, Planning, Environment, Balance of Nature, Management

DILUTION TABLE

Concentration desired			To prepare solutions of concentration indicated at left, take number of milliliters of stock solution shown below, and make up to one liter with suitable dilution water.					
%	ppm or mg/l	ppb or µg/l	Stock sol: 1% 10 gm/l	Stock sol: .1% 1 gm/l	Stock sol: .01% 1 gm/l	Stock sol: .001% .01 gm/l	Stock sol: .0001% .001 gm/l	Stock sol: .00001 % .0001 gm/l
100.	1,000,000							
10.	100,000							
1.0	10,000		1000					
.56	5,600		560					
.32	3,200		320					
.18	1,800		180					
.1	1,000		100	1000				
.056	560		56	560				
.032	320		32	320				
.018	180		18	180				
.01	100		10	100	1000			
.0056	56		5.6	56	560			
.0032	32		3.2	32	320			
.0018	18		1.8	18	180			
.001	10		1.0	10	100	1000		
.00056	5.6			5.6	56	560		
.00032	3.2			3.2	32	320		
.00018	1.8			1.8	18	180		
.0001	1.0	1000		1.0	10	100	1000	
.000056	.56	560			5.6	56	560	
.000032	.32	320			3.2	32	320	
.000018	.18	180			1.8	18	180	
.00001	.10	100			1.0	10	100	1000
.0000056	.056	56				5.6	56	560
.0000032	.032	32				3.2	32	320
.0000018	.018	18				1.8	18	180
.000001	.010	10				1.0	10	100
.00000056	.0056	5.6					5.6	56
.00000032	.0032	3.2					3.2	32
.00000018	.0018	1.8					1.8	18
.0000001	.0010	1.0					1.0	10
.000000056	.00056	.56						5.6
.000000032	.00032	.32						3.2
.000000018	.00018	.18						1.8
.00000001	.0001	.10						1.0

Originally prepared for Training by C. Henderson; modified and arranged by H. W. Jackson.

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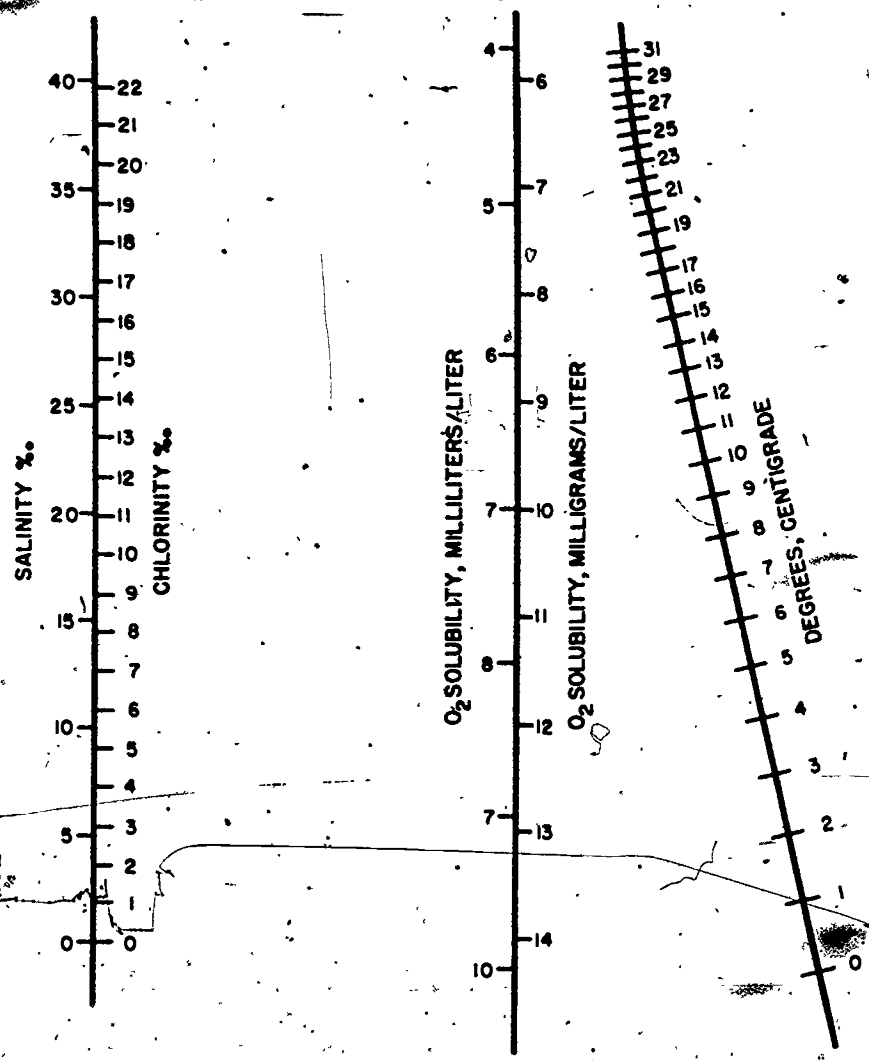


NOMOGRAM FOR THE SOLUBILITY OF OXYGEN

RICHARDS AND CORWIN, LIMNOLOGY AND OCEANOGRAPHY, 1956

USE OF LC__ PAPER

NOTE: The "LC__ Paper" has been especially designed for use in the Training Program of Water Programs Operations. (It is not known to be commercially available.) The same results can be obtained with conventional graph paper (two-cycle semi-log is recommended).

I INTRODUCTION

A Measurements of Toxicity

Bioassay results using fish are expressed in terms of Tolerance Limits (LC) for time "t". The percentage of experimental animals surviving for the specified period of time is written as a subscript to the LC symbol. For example, the "96-hour LC_{50}" is that concentration of a substance which 50% of the experimental fish can tolerate for 96 hours. The LC_{50} is equivalent to the median tolerance limit (LCm).

The use of LC with the percentage subscript allows the designation of percentage survivals other than 50%; e.g., a $LC_{10}{}^{t}$ would indicate that only 10% of the fish could tolerate a given concentration for time t, while a $LC_{90}{}^{t}$ would indicate a concentration which could be tolerated by 90% of the fish for the time specified. The LC_{50} is currently the standard and should always be determined.

Unless specified to the contrary, the laboratory exercise in this training course will concentrate on the determination of LC_{50}'s, and the instructions below are so written.

B Preliminary Procedures

Examine the "control" container. A bioassay test should not be accepted as reliable unless at least 90% of the control animals survive. Death of any of the controls should be clearly explained in the "Notes" at the right. If control survival is satisfactory, proceed as follows (if not, repeat the test).

II PREPARATIONS FOR CALCULATING A LC_{50}

A Fill in preliminary information as called for on the right side of the sheet, including the subscript "50" in the title and also in the box after "Final Results"; and the time intervals to be employed; e.g., 15 min., 1 hr., 4 hrs., or 24 hrs., 48 hrs., 96 hrs., etc. (These are the Time "t's"). Circle the term in which the experimental concentrations are expressed; fill in the name of the test species, the temperature range, and describe the dilution water. Any number of LC_m's may be calculated from a given setup at successive time intervals.

B Insert decimal points and/or zeros in the column of numerals above "Bioassay Concentrations" to represent the dilutions actually used in the test. If the series used does not fit the lines provided, use the coordinate LC__ Paper, or request further instructions.

III TO ESTIMATE THE LC_{50} AT TIME "T"

A Find the "Percent Survival" scale at the bottom of the graph. Indicate the percent survival at each of the concentrations tested. Use a code to mark the points at successive times as: a tiny circle, a triangle, a square, or a color code.

B Locate the highest test concentration showing greater than 50% survival. Connect this point to the survival percentage of the next highest concentration with a straight line.

C Read on the scale at the left, the value of the point where the above line and the 50% survival line intersect. This value is the LC_{50} concentration for the time interval in question.

D If there are points below (i.e., at lesser concentrations) which show less than 50% survival, an unreliable population of experimental animals, poor handling, or other detrimental factor may be indicated. Re-examine the survival of the controls. If it is less than 100%, consider the advisability of repeating the test.

IV COMMENTARY

The $LC_{50}{}^{t}$ concentration is that which will permit half of the experimental organisms to survive for time "t" under the conditions of the test. If by chance one of the experimental concentrations happened to have 50% survival at time "t" that is the LC_{50} concentration, no further calculation is necessary (provided there is no higher concentration which showed an equal or higher survival rate).

This outline was prepared by H. W. Jackson, Chief Biologist, National Training Center, WPO, EPA, Cincinnati, OH 45268.

SEC 151
(6-58)

BIOASSAY RECORD SHEET

Series: ____________ Company: ______________________________ Date: ________

Technician: ______________________________ Starting Hour ________

Material being tested: ______________________________

Source: ______________________________

Source of dilution water: ______________________________

Test species ______________________________ Temp. range: ________

No. individuals per concentration: ______________________________

Start									
Dilution:									Control
DO									
pH									
Hardness									
Other									

24 hours									
No surviving									
% survival									
DO									
pH									
Other									

48 hours									
No surviving									
% survival									
DO									
pH									
Other									

96 hours									
No surviving									
% survival									
DO									
pH									
Other									

BI. BIO. met. 9c. 11. 69

SEC 151
(6-58)

BIOASSAY RECORD SHEET

Series: ______ Company: ______ Date: ______

Technician: ______ Starting Hour ______

Material being tested: ______

Source: ______

Source of dilution water: ______

Test species ______ Temp. range: ______

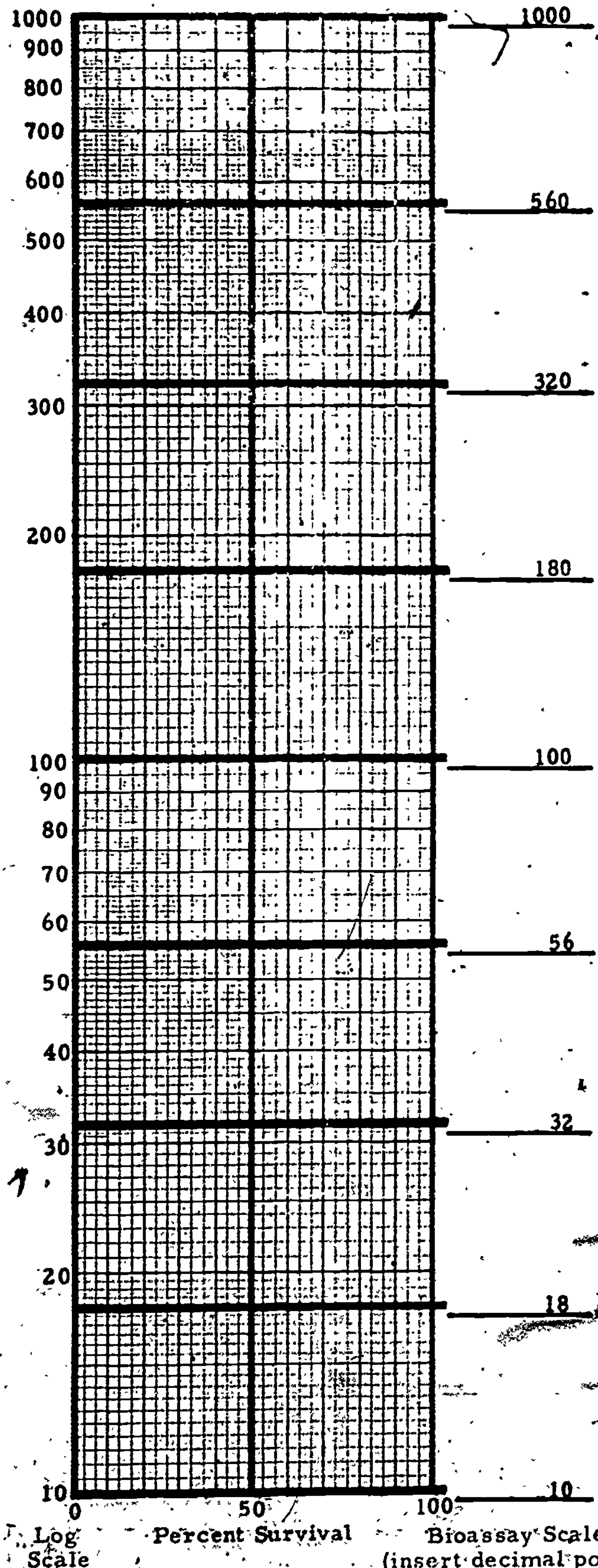
No. individuals per concentration: ______

Start									
Dilution:									Control
DO									
pH									
Hardness									
Other									
24 hours									
No surviving									
% survival									
DO									
pH									
Other									
48 hours									
No surviving									
% survival									
DO									
pH									
Other									
96 hours									
No surviving									
% survival									
DO									
pH									
Other									

BI. BIO. met. 9c. 11. 69

LC PAPER

Sheet No. or Code: ________



Material Tested: ________________________

____

Starting Date: ________ Hour: ________

Final Results:

	Time	Intervals		
LC				

Concentrations Expressed as (circle one):

%, mg/l, other: ________________________

Test species: ________________________

Temperature Range: ________________________

Dilution Water (source & characteristics):

Notes: ________________________________

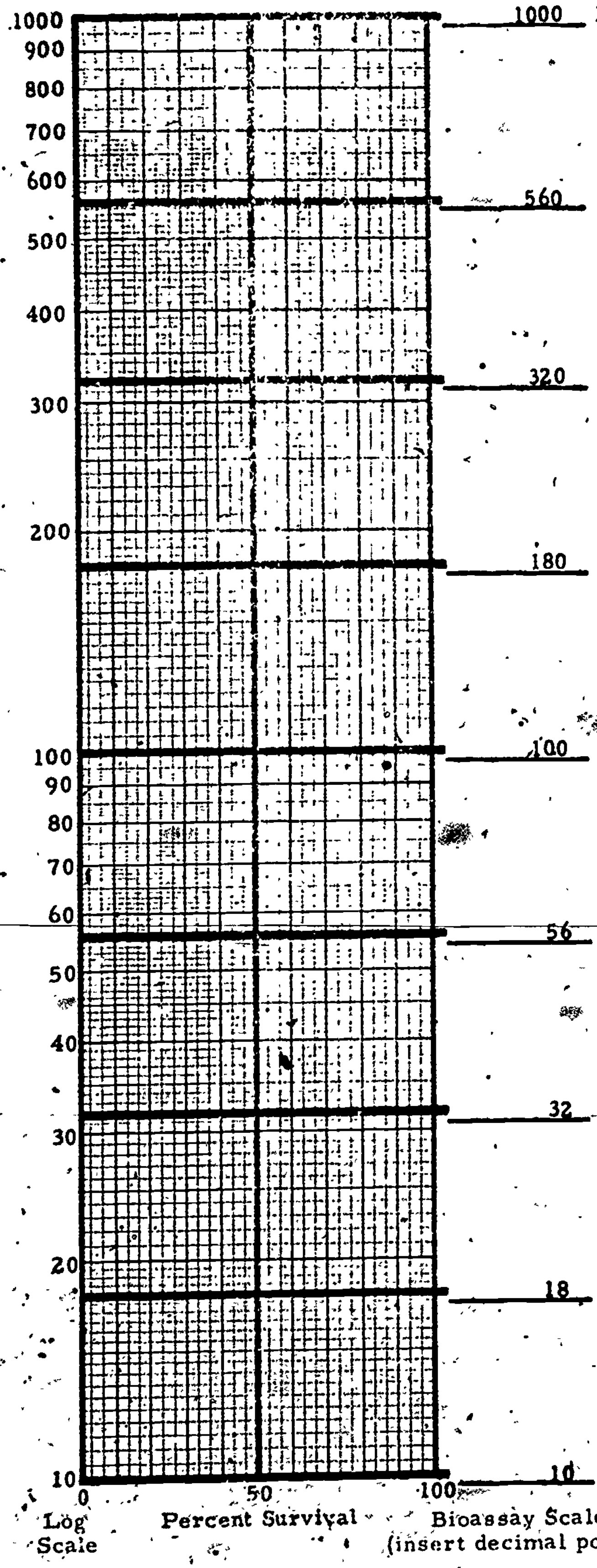
Technician: ________________________

Note: This paper not commercially available.

BI. BIO. met. 15b. 6. 73

LC PAPER

Sheet No.or Code:________



Material Tested:________________________

____

Starting Date: ________ Hour:

Final Results:		Time Intervals		
LC				

Concentrations Expressed as (circle one):

%, mg/l, other:________________

Test species:________________

Temperature Range:________________

Dilution Water(source & characteristics):

Notes:________________________

Technician:________________________

Note: This paper not commercially available.

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SEC 187
(Rev. 11-69)

LC_m PAPER
(Coordinate)

Sheet No. or Code: ____________

Material Tested: ____________

____

Starting Date: Hour:

Final Results:

LC	Time Intervals		

Concentrations Expressed as (circle one):

%, mg/l, other: ____________

Test species: ____________

Temperature Range: ____________

Dilution Water (source & characteristics):

____

Notes: ____________

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Technician: ____________

0 50 100

Scale Percent Survival Bioassay Concentrations

Note: This paper not commercially available:

BI.BIO.met. 16a. 11.69

SEC 187
(Rev. 11-69)

LC_m PAPER
(Coordinate)

Sheet No. or Code: ____________

Material Tested: ____________

____

Starting Date: ____________ Hour: ____________

Final Results:

	Time Intervals		
LC			

Concentrations Expressed as (circle one):

%, mg/l, other: ____________

Test species: ____________

Temperature Range: ____________

Dilution Water (source & characteristics):

____

Notes: ____________

Technician: ____________

0 50 100

Scale Percent Survival

Bioassay Concentrations

Note: This paper not commercially available.

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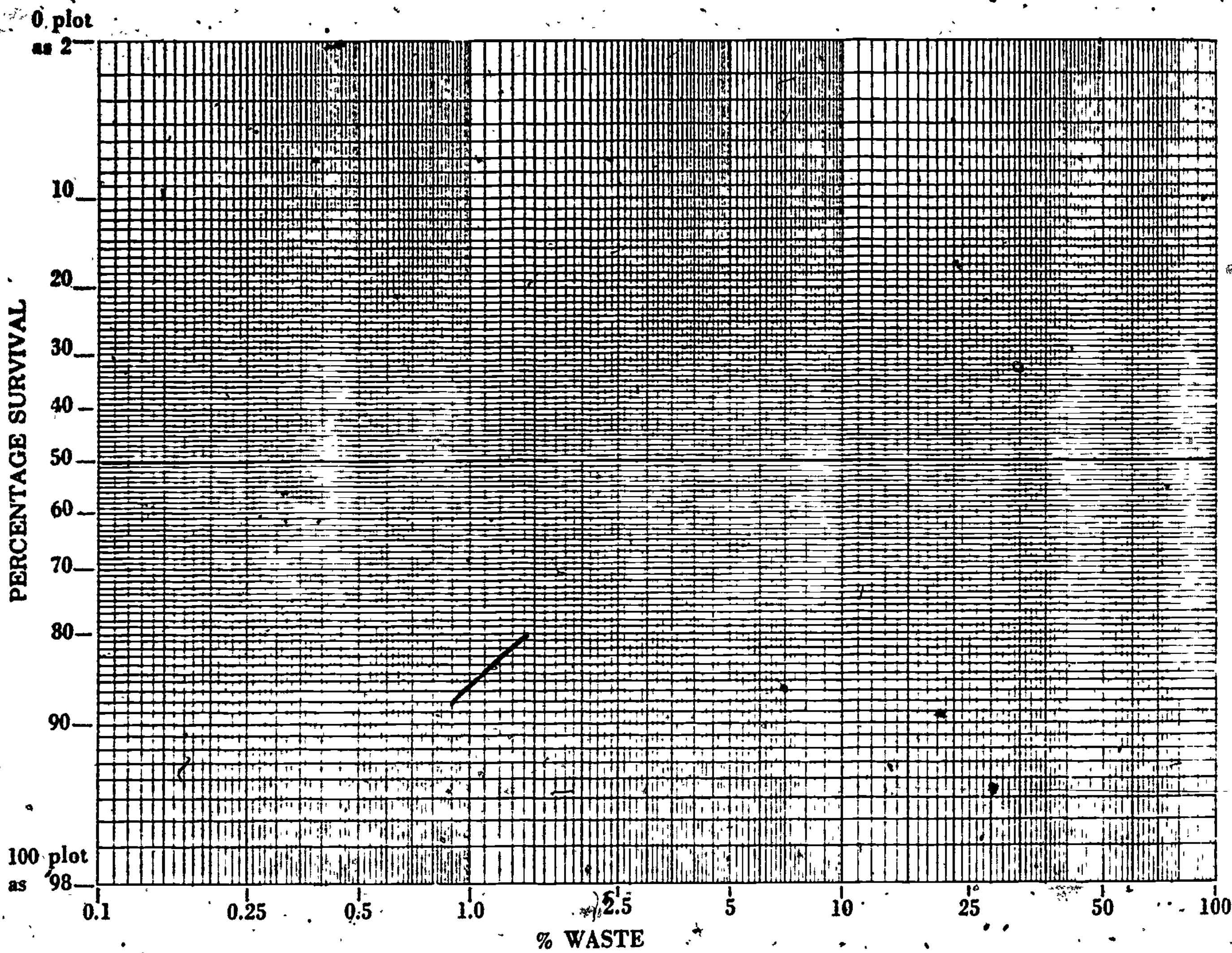
(Extracted from: ORSANCO 24-Hour Bioassay, January 1974)

BIOASSAY PAPER (Log-probit) Code: ________



Material Tested: ________

Starting Date: ________ Hour: ________

Concentrations expressed as (circle one): %, mg/liter, ________

Test species: ________ Temperature: ________

Dilution water source and characteristics: ________

Other notes: ________

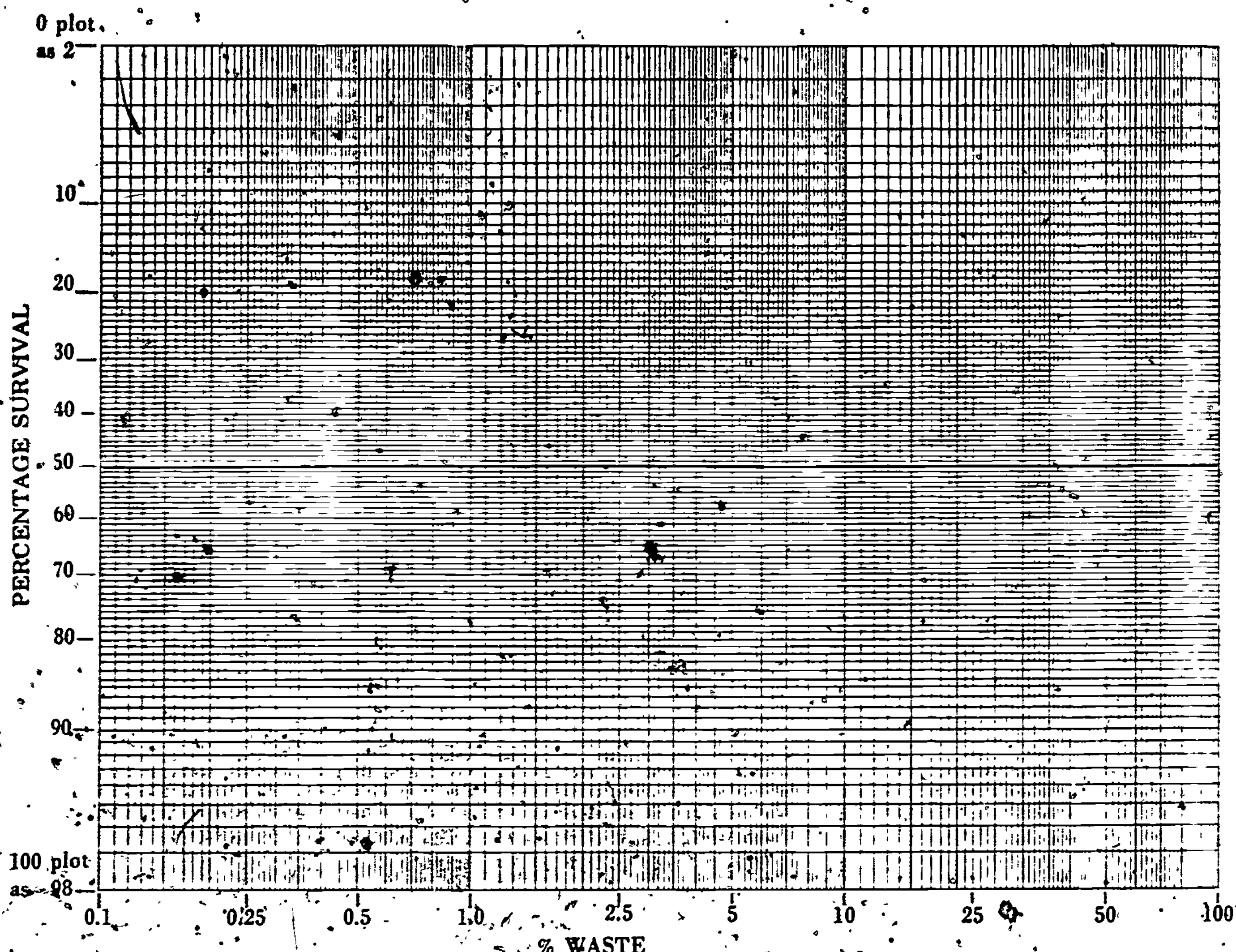
Final Results:

Time interval		24 hr.	48 hr.	96 hr.	
LC50					

Observer: ________

(Extracted from: ORSANCO 24-Hour Bioassay, January 1974)

BIOASSAY PAPER (Log-probit) Code:________



Material Tested:________

Starting Date:________ Hour:________

Concentrations expressed as (circle one): %, mg/liter,________

Test species:________ Temperature:________

Dilution water source and characteristics:________

____

____

Other notes:________

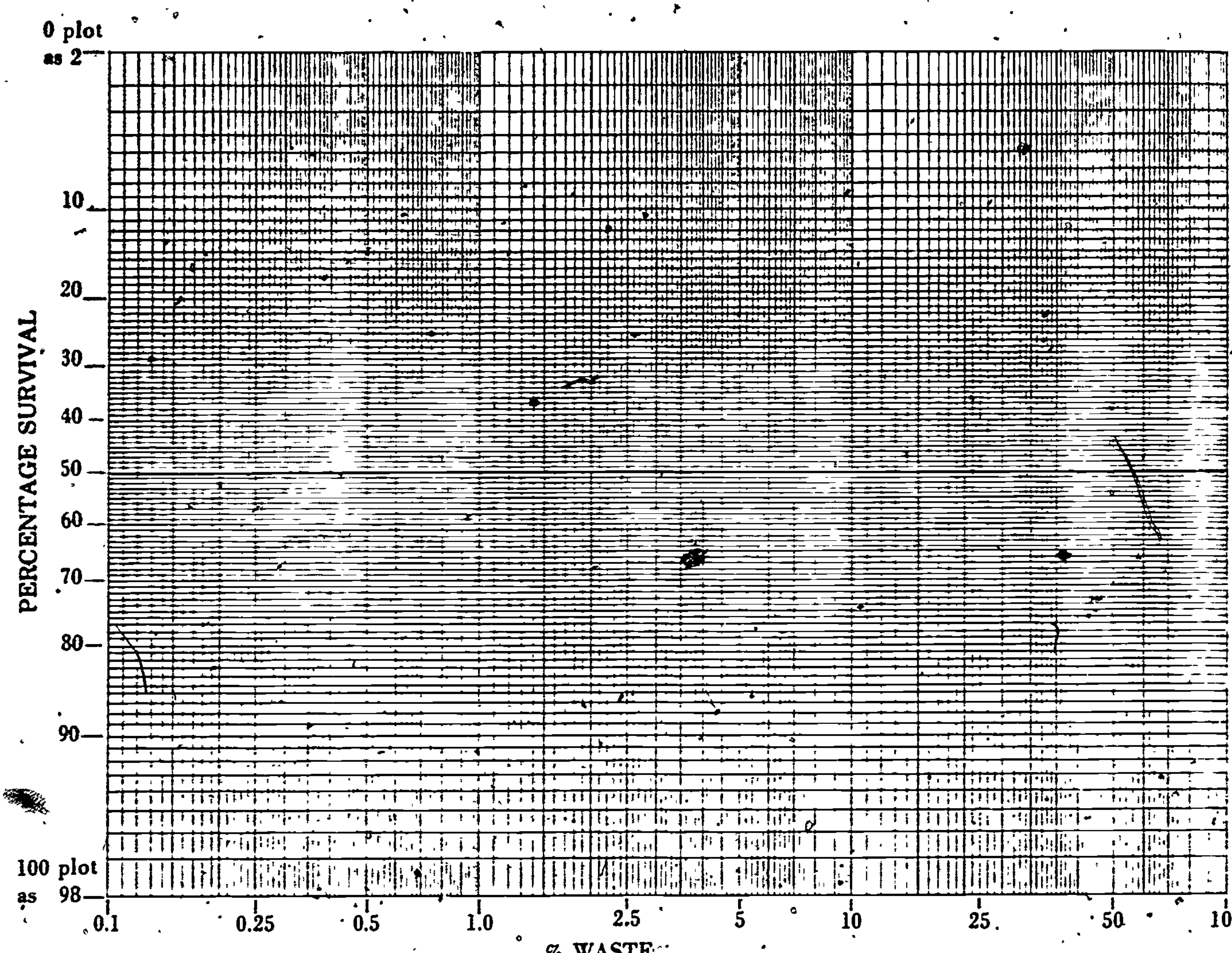
____

Final Results	Time interval		24 hr.	48 hr.	96 hr.	
	LC50					

Observer:________

(Extracted from: ORSANCO 24-Hour Bioassay, January 1974)

BIOASSAY PAPER (Log-probit) Code:________



Material Tested:________

Starting Date: ________ Hour: ________

Concentrations expressed as (circle one): %, mg/liter, ________

Test species:________ Temperature:________

Dilution water source and characteristics: ________

Other notes: ________

Final Results:

Time interval		24 hr.	48 hr.	96 hr.	
LC50					

Observer:________