

Procedures for
INVESTIGATION OF FISH-KILLS

A Guide for Field Reconnaissance and Data Collection

Prepared by the
AQUATIC-LIFE ADVISORY COMMITTEE
of the
OHIO RIVER VALLEY
WATER SANITATION COMMISSION

OHIO RIVER VALLEY WATER SANITATION COMMISSION

An interstate agency representing Illinois, Indiana, Kentucky, New York, Ohio, Pennsylvania, Virginia and West Virginia.

MEMBERS

ILLINOIS

ROLAND R. CROSS, M.D.
Director of Public Health

CLARENCE W. KLASSEN
Chief Sanitary Engineer

MAURICE E. GOSNELL
Lawrenceville, Illinois

INDIANA

A. C. OFFUTT, M.D.
State Health Commissioner

B. A. POOLE
Technical Secretary
Stream Pollution Control Board

JOSEPH L. QUINN, JR.
The Hulman Company

KENTUCKY

RUSSELL E. TEAGUE, M.D.
State Health Commissioner

EARL WALLACE
Division of Game and Fish

LABAN P. JACKSON
Commissioner of Conservation

NEW YORK

JOSEPH R. SHAW
President, Associated Industries
of New York State, Incorporated

HERMAN E. HILLEBOE, M.D.
State Health Commissioner

EARL DEVENDORF
Department of Health

OHIO

HUDSON BIERY
Terrace Park, Ohio

KENNETH M. LLOYD
Executive Secretary
Mahoning Valley Industrial Council

RALPH E. DWORK, M.D.
Director of Health

PENNSYLVANIA

E. A. HOLBROOK
Pittsburgh, Pennsylvania

H. E. MOSES
Department of Health

BERWYN F. MATTISON, M.D.
Secretary of Health

VIRGINIA

E. BLACKBURN MOORE
Chairman, Water Control Board

T. BRADY SAUNDERS
Commissioner, Water Control Board

ROSS H. WALKER
Commissioner, Water Control Board

WEST VIRGINIA

N. H. DYER, M.D.
State Health Commissioner

W. W. JENNINGS
State Water Commission

HARRY K. GIDLEY
Charleston, West Virginia

UNITED STATES GOVERNMENT

O. LLOYD MEEHEAN
Fish and Wildlife Service

LEONARD A. SCHEELE, M.D.
Surgeon-General
Public Health Service

EDWIN E. ABBOTT
Corps of Engineers

OFFICERS

EARL DEVENDORF
Chairman

F. H. WARING
Secretary

KENNETH M. LLOYD
Vice-chairman

VERNA B. BALLMAN
Treasurer

EDWARD J. CLEARY
Executive Director

LEONARD A. WEAKLEY
Legal Counsel

Procedures for INVESTIGATION OF FISH-KILLS

A Guide for Field Reconnaissance and Data Collection

Prepared by the
AQUATIC-LIFE ADVISORY COMMITTEE

- DR. L. L. SMITH, JR.; Department of Entomology and Economic Zoology, University of Minnesota (Chairman)
- DR. B. G. ANDERSON; Head, Department of Zoology and Entomology, The Pennsylvania State University
- DR. W. A. CHIPMAN; Chief, Special Shellfishery Investigations, Fish and Wildlife Service
- DR. JAMES B. LACKEY; Department of Civil Engineering, University of Florida
- DR. O. L. MEEHEAN; Assistant to the Director, Fish and Wildlife Service
- DR. EDWARD SCHNEBERGER; Superintendent, Fish Management Division, Wisconsin Conservation Commission
- DR. W. A. SPOOR; Department of Zoology, University of Cincinnati
- DR. C. M. TARZWELL; Chief of Aquatic Biology, Robert A. Taft Sanitary Engineering Center, Public Health Service
- Commission liaison and secretary—
W. G. HAMLIN, Ohio River Valley Water Sanitation Commission

of the
**OHIO RIVER VALLEY
WATER SANITATION COMMISSION**

March, 1956

Price 50 cents

FOREWORD

A special responsibility rests upon water-pollution control agencies to deal promptly and effectively with the investigation of a fish-kill. Quite often—but not always—a fish-kill is the unfortunate result of a rapid change in water-quality. This is a prime reason for commanding immediate attention by regulatory authorities. But there is a public relations aspect that is equally compelling. Many people have been sensitized to the evils of pollution; and fish-kills are a visible manifestation that something is wrong. It is important, therefore, for the administrative agency to quickly ascertain the facts, announce its findings and, where corrective measures are indicated, give assurance that these have been instituted.

This handbook had its origin in the desire of the eight states signatory to the Ohio River Valley Water Sanitation Compact—Indiana, Illinois, Ohio, Virginia, New York, Pennsylvania, West Virginia and Kentucky—to perfect their practice in the investigation of fish-kills. A fish-kill investigation is a specialized form of endeavor. The conditions resulting in a fish-kill often are dynamic, and field investigations not only must be launched with dispatch but should be organized to secure all the data necessary for a proper appraisal of cause.

This desire for a practical outline of what to do and how to do it was transmitted to the Aquatic-Life Advisory Committee of the Commission. This committee, whose membership comprises a group of national authorities in fisheries science and management, was organized by invitation of the Commission in 1952. Among its contributions to advancing the work of the Commission, all of which are being rendered as a public service, was the compilation of this handbook.

EDWARD J. CLEARY
*Executive Director
and Chief Engineer*

CONTENTS

FISH KILLS	<i>Page</i> 5
Causes of Mortality	5
Importance of Prompt Investigation	6
INVESTIGATION OF KILLS	7
Extent and Duration of Kill	8
Physical Conditions	9
Chemical Conditions	10
Biological Information	11
Sources of Pollution	13
PLANNING FIELD PROCEDURE	15
Initial Report	15
The Field Plan	15
REPORTS OF FINDINGS	16
APPENDIX A — Judging Toxicity by Bioassay.....	18
APPENDIX B — Selected Bibliographies	21
Bioassays and Toxicity	21
Biological Indicators of Pollution	23

Photo credits — Page 4, Wisconsin Conservation Department — Page 7, Robert A. Taft Sanitary Engineering Center, Public Health Service — Page 11, Minnesota Department of Health — Page 12 (top left), Robert A. Taft Sanitary Engineering Center, Public Health Service — Page 12 (top right and bottom), Minnesota Department of Health — Page 17, Robert A. Taft Sanitary Engineering Center, Public Health Service.



Collection of essential information is facilitated by the use of a field kit equipped for determination of dissolved oxygen, pH, carbon dioxide, alkalinity and acidity.

FISH-KILLS

Fish-kills in natural waters can cause serious depletion of fish populations and usually create administrative problems. Prompt investigation and efficient handling of the collected data are essential to effective water and fishery management.

This discussion of fish-kills and the outlined procedure provide a uniform practice for dealing with the initial field investigations of these problems and are not intended as a recommendation for general pollution surveys. Where there are known problems that have been under observation, or when specialists trained in the chemical and biological aspects of waste toxicity are immediately available to study kills, these procedures may be modified according to the judgment of the investigator in charge.

CAUSES OF MORTALITY

Natural fish-kill—The public generally assumes that fish-kills are the result of negligence on the part of: (1) individuals, (2) industries, or (3) communities. Many kills, however, result from natural causes which may or may not be correctable. It is important, therefore, that everyone investigating causes of fish-kill be thoroughly aware of the numerous possible causes of mortality.

Among the natural causes in northern areas are winter kills. These kills are usually observed shortly after the ice goes out in the spring and may be of greater or less extent depending upon the severity of the oxygen depletion which has been the primary cause of the kill.

A second cause of natural kill is overnight oxygen depletion associated with plant respiration and decay during the summer months, especially in periods of very warm weather. This condition may arise as a result of both algal and higher-plant growths and may be aggravated by increased fertility from organic pollution. Occasionally the kill may be caused by release of toxins from decaying algae.

A third cause of mortality may be natural temperature changes. Usually these conditions arise where long periods of very warm weather result in the raising of stream or lake temperatures above the lethal levels for some particular cold water species or, in a few cases, for all species.

A fourth cause of natural fish mortality is epidemic disease or endemic disease, parasites and natural death at spawning time.

A fifth and occasional cause is lake inversion during vernal-autumnal turnover, which results in toxic materials or deoxygenated water being brought to the surface.

Kills from mechanical agents—Mechanical actions of various types may cause fish mortality. These are usually associated with some human activity and can be observed directly or by their effect on the bodies of the fish.

Commonest among them are explosion and abrupt water fluctuations through control of dams that leave fish stranded. Unscreened irrigation ditches or natural drainage ditches, which may influence fish migration after rains or seasonal runoffs, may also trap fish and result in a fish-kill.

A third but unusual cause of fish-kill from mechanical means is associated with dredging, mining, construction activity, or other disturbance of the stream bed that makes the water so turbid that a kill results. Kills may also be caused by the action of pumps, turbines, spillways or strong winds.

Kills caused by polluting substances—Fish-kills resulting from polluting substances other than inert materials are usually caused either by materials with high oxygen demands that remove all oxygen from the habitat or by toxic materials that actually poison the fish. Heated water from industrial sources may also cause mortality.

IMPORTANCE OF PROMPT INVESTIGATION

In view of the numerous possibilities, all potential causes of fish-kill must be investigated and none should be ignored until it has been eliminated for good reason. It is important that all possible speed be exercised in conducting the initial phases of any fish-kill investigation since dead fish disintegrate rapidly and the immediate causes of death may disappear or become unidentifiable within a few hours.

A thorough investigation is important from both the standpoint of public relations and possible legal action. If the death of fish is caused by natural agencies beyond human control, the public should be so informed in order that possible sources of industrial or sewage waste discharges are absolved from responsibility. If, on the other hand, there is possible legal liability, the investigation must be made in such a way that appropriate action can be taken. In such cases the necessity for securing reliable data cannot be over-emphasized.

INVESTIGATION OF KILLS

The following outline is to be used only as a general guide to administrators and others who have the responsibility of organizing field investigations of fish-kills and evaluation of results. No attempt has been made to specify techniques. All sampling and testing procedures for securing physical and chemical data should be those indicated in "Standard Methods for the Examination of Water and Sewage," 10th Ed. Amer. Pub. Health Assn., except when other generally approved methods for special problems will be more useful. Biological procedures should follow accepted practice in the respective fields.

It is assumed that the data will be appraised by scientists or administrators competent to do so. Therefore, no suggestions for interpretation of results are included. The procedures are intended to cover emergency situations associated with temporary or acute problems which result in fish mortality. *Where continued pollution with potentially toxic or otherwise harmful effects is involved, more detailed and long range observations must be developed for each situation.*

Collection of samples and inspection of streams too deep for wading requires a boat in which a field kit for chemical determinations can be used.



The personnel available for investigations will vary, of course, with location and organization of local conservation and pollution-control agencies, but in general will be of five types:

1. The lay observer with no technical training but with local interest and local knowledge;
2. The conservation officer or fishery manager who has varying degrees of skill, but who can be relied upon for accurate descriptions of time, geographical extent of kill, numbers and usually species of fish involved, and potential sources of pollution, and who may be equipped to take some samples such as dissolved oxygen;
3. The field biologist who is competent to handle all phases of the field investigations;
4. The specialized technician, engineer, or laboratory man capable of handling special chemical, engineering, or biological problems who can be called in after the nature of the problem is defined; and
5. The supervisor or administrator who can appraise results and initiate appropriate actions.

The following descriptions of data to be collected indicate the type of personnel suited for a particular phase of the work. *It is essential that all work requiring technical skill or judgment be performed by trained biologists or other specialized personnel.*

EXTENT AND DURATION OF KILL

Time, duration and extent of kill—The time when fish-kill started and when it ended should be carefully determined from local observers or from direct observation by the investigator. If the kill is acute, and occasional, this will probably be relatively easy. If it is a case of chronic mortality, time determinations will be more difficult; but effort should be made to ascertain whether a kill is continuous, at intermittent periods of the day, on alternate days or some other time interval.

The area of fish-kill should be carefully ascertained, especially in the case of streams where fish may wash away from the location of kill. In streams this can be done with reference to bridges, dams or industrial plant locations. If the area of kill extends over considerable mileage it is best to define it by reference to legal descriptions of adjacent land or political subdivision.

Number of fish affected—The number of fish killed is hard to estimate since all are not observable or they may be present in such numbers that it is impractical to attempt to count them. It is important, however, to note the difference between true fish-kill and accumulated natural mortalities that sometimes collect in numbers on a lee beach after a wind. It is suggested that the number of fish per running foot of beach or shore line be counted where practical, or that estimates be made on an area basis where the number of fish cannot be counted because of excessive quantities.

Species killed—All dead fish should be identified by common name and scientific name. When initial observers do not know scientific names, the biologist should determine exact species as soon as he arrives at the site of kill.

Size range of species—An appraisal of the size-range of each species killed should be made, and with it observations on the predominance of particular size groups if distribution appears to be other than random.

Supplementary statements—It is desirable to note the names of lay observers who have reported the kills or given other information, together with a detailed report of their observations and a signed statement if possible. This information may be useful in assisting the professional observer to arrive more quickly at the cause of trouble and also is useful in later legal actions or public relations work in connection with the problem.

Personnel—Lay observers may be used for reporting kill, time of kill and supplementary data. A conservation officer, fishery manager, or fishery biologist will report number, species, size range and numbers of fish killed, depending on the location of individuals and the administrative setup of the responsible agencies.

PHYSICAL CONDITIONS

Temperature—Water-temperature fluctuation is a possible direct or contributing cause of mortality. Temperature should be recorded over a series of hours especially during the hottest period of the day. It is suggested that hourly observation during the daylight hours be made and that several readings be taken during the night. If artificial sources of warm water are contributing to the temperature problem, a temperature reading should be taken every hour throughout a 24-hour period. Particular previous knowledge of temperature problems may suggest specific timing of observations in individual cases.

Water flow—Observation of flow in streams should be made, noting especially whether it is constant or intermittent as would be the case with hydro-electric power operations. The pattern of stream flow and excessive or abnormal waste flow preceding kill is to be determined where possible. Records of hydro-electric developments and the U. S. Geological Survey data should be consulted where available.

Weather preceding kill—Local precipitation, wind, sunlight and air temperature for the two or three days preceding observation of dead fish should be determined. These data usually can be collected from weather bureau sources but if this is not possible, observation by local people or newspaper reports are helpful.

Personnel—These investigations are to be carried on by field biologists who may secure some of their data from other reliable observers.

CHEMICAL CONDITIONS

It is important that chemical data be collected at various stations throughout the area of kill and from possible pollution sources. In a stream at least one sample should be taken above the source of pollution and one below the zone of recovery—the area where stream conditions are returning to those normal for the stream. The exact number of stations can be determined only by a competent technical observer who has surveyed the situation.

Dissolved oxygen—Dissolved-oxygen observations should be made periodically during a 24-hour period in every water where oxygen depletion is a possibility. If there is any reason to suspect diurnal fluctuation of appreciable magnitude, observations should be made at one-hour or other closely spaced intervals throughout the 24-hour period, with observation times so spaced that sunrise will be bracketed between two observations.

Biochemical oxygen demand—In some cases it may be desirable to make B.O.D. determinations. They should be made from samples collected and analyzed in accordance with Standard Methods where problems of oxygen deficiency are suspected and when proper handling can be insured. If there appears to be intermittent discharge of pollutants, the B.O.D. determinations must be made throughout the 24-hour period at intervals indicated by the local situation. Immediate oxygen demand should be determined where general conditions suggest utility of this technique.

Hydrogen-ion concentration—Since changes in pH due to natural causes or to introduction of acidic or basic wastes can cause fish-kills, a series of observations on a 24-hour basis should be made.

Toxic pollutants—Samples of water for chemical analysis should be collected from stream and pollution sources in chemically cleaned bottles and analyzed for suspected toxic substance in accordance with standard practice in central laboratories. When practical, preliminary field analyses for possible toxic materials may be made. In cases where well-managed industries may be a potential source of harmful waste, they may keep complete records of materials discharged and of stream conditions. If such records can be obtained they will materially aid the investigator.

Personnel—Field biologist and laboratory technicians will be required for these observations.

BIOLOGICAL INFORMATION

Fish samples—In many cases it will be difficult to collect fish samples for further examination and often impossible to make significant findings from autopsy. Unless fish are actually seen dying and are preserved immediately by icing or sharp freezing, they will be of little value in determining causes of death, except to the extent that they will indicate parasites or disease. If fish

Variation in numbers and types of plankton provide an important clue to changes in stream conditions. Here the plankton "catch" is being washed from the net into the sample collector.





Three types of bottom samplers for streams. A Surber net sampler is shown at top left. The serrated edge of the circular type sampler (top right) is an advantage in cutting into clay bottoms. A Peterson dredge appears in the lower photo, in position to be lowered for a bottom sample.



are collected, they should be shipped promptly to the nearest laboratory where competent examination can be made.

Plant and algal samples—It is important to make collections of algae where this plant material is present in quantities and to make careful observations of the extent and density of rooted aquatic plant growth. These observations are especially important in lakes or large rivers where natural mortality from overnight oxygen depletion or toxic products of decay are a possibility.

Macro-invertebrates—Samples of bottom-living macro-invertebrates should be collected from areas of suspected pollution, especially where toxic wastes are involved, since they offer a good index to source and extent of a toxic pollutant.

Use of test fish—Where practical, and when observations indicate continued toxicity of water, holding test fish of appropriate species in wire baskets at various distances downstream from suspected sources of toxic effluent may assist in locating the true source and delimit areas where toxic effects occur.

Bioassay—If facilities and personnel for conducting bioassay are available, adequate quantities of water from areas of fish-kill and suspected effluents should be collected and taken to a laboratory immediately.

Personnel—These observations and laboratory analysis should be made only by a competent biologist or, in the case of autopsy, by appropriate specialists. Conservation officers or fishery managers may collect fish and freeze them for further reference.

SOURCES OF POLLUTION

All possible sources of pollution should be investigated in connection with every fish-kill. In addition to enumerating the sources of pollution an effort should be made to ascertain the volume of pollutant, the nature of the wastes and the regularity of flow. These data may be available from the industry, disposal plant or state pollution control agency. Among the sources to be considered are:

Temporary activities—These include crop dusting, mosquito-control operations, chemical treatment of construction materials adjacent to lake or stream and other operations that are temporary in nature.

Industrial activities—Industrial activities represent one of the most common sources of deleterious pollution. Discharge from any industrial operation should be noted and reported carefully.

Domestic sewage—The important sources of domestic pollution are municipal systems or other community disposal facilities. Individual homes or establishments with limited population rarely cause pollution of sufficient magnitude to be important in fish-mortality problems.

Agricultural activity—Certain agricultural activities may contribute to fish-kill, especially where there is extensive use of fertilizers that may wash into the streams, or where the disposition of manure is such that it can drain into a stream and thereby cause an increase in the biochemical oxygen demand.

Personnel—Field biologists, sanitary engineers or other specialists may collect these data. Lay observers and conservation personnel may have information on sources of pollution from knowledge of local activities.

Field investigations should be pointed toward locating all potential sources of pollution. Here is an example of an unsuspected discharge that occurred from efforts to relieve a temporarily overloaded industrial waste lagoon.



PLANNING FIELD PROCEDURE

A carefully developed plan of routine field procedure should be available for immediate activation when an emergency is reported.

THE INITIAL REPORT

The initial report of a fish-kill usually comes from a lay observer to a game warden or fishery manager. Sometimes it is transmitted directly to the headquarters of the conservation department or pollution-control agency. If the report is from a lay observer, the nearest available conservation officer, fishery manager, fishery biologist, pollution-control biologist or sanitary engineer should be dispatched to the scene of the kill.

If initial observations are made by a conservation officer or fishery manager, he should immediately contact the appropriate technical personnel when he has determined that an actual fish-kill has occurred or is in progress. Placing responsibility on the local officer for an initial report by phone will eliminate false alarms where only slight natural mortality has been observed and thus save time and expense of sending specialists to the scene. Exact procedure will depend on the structure of the local organization.

In states where administrative lines permit, the entire investigation should be channelled through one agency with predetermined agreement on cooperation from associated agencies.

THE FIELD PLAN

The plan of field investigation should be based on the following sequence and should be carried out by the appropriate field officer or technician as indicated in previous section on collection of data:

1. Extent of area where kill occurred and where fish were found.
2. Amount of fish-kill (species, size, numbers and weight).
3. Collection of biological data as previously defined, including study of sources of pollution, and a sample of fish killed for evidence in the event that litigation follows.

4. Collection of B.O.D. samples and shipment to laboratories.
5. Collection of samples for laboratory water analysis, for field analysis of toxic materials and for bioassay.
6. Collection of temperature, dissolved oxygen and pH observations over entire area on a 24-hour basis where indicated.
7. Collection of samples from suspected pollution sources. (Items 5, 6 and 7 should be carried on simultaneously where the kill appears traceable to a definite pollution source.)
8. Investigation of pollution sources. This appraisal should include an enumeration of all possible sources, reports of interviews with interested parties and checking of industrial plants and processes. This information can be secured after all the preliminary observations have been carried out.

REPORTS OF FINDINGS

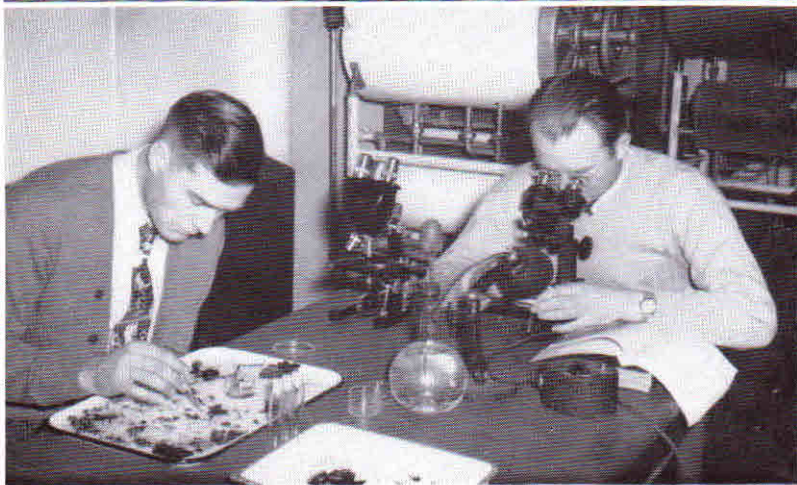
The reports of findings in fish-kill investigations should be made only by competent biologists thoroughly qualified to judge the nature and completeness of the data collected. Recommendations should be included only with the sanction of, and review by, administrative officers of conservation or pollution-control agencies.

The final reports should be such that they can be introduced as evidence into court and will stand scrutiny and cross-examination. Speculation or unsubstantiated hypotheses must not be included except in the form of suggestions for further observations. It is desirable that field and laboratory data sheets be maintained in a form that can be submitted in court if such records are required. Where calculations are made on the basis of limited observations they should be appraised on the basis of their statistical validity. Simple averages of observed conditions should not be considered definitive where variability is great unless justified by appropriate statistical analysis.

When samples are collected in the field and returned to a central laboratory for examination, they should be sealed and identified by the sender so that in case of litigation the chain of evidence will remain intact. In the laboratories samples should be opened with due regard for this maintenance of identity.

When the final reports are prepared, they should be filed with the agency authorizing the investigation which will, at its discretion, make them available to other governmental agencies concerned, polluters and interested civic groups where such procedure will be in the public interest. When legal action is indicated, the investigating agency should place the report in the appropriate hands for such consideration.

Laboratory examinations supplement the field investigation. At the top is shown the tanks in a constant temperature room for maintaining stock fish to be used in bioassay tests. (Bottom) Organisms recovered from a bottom sample are being separated for counting and identification.



APPENDIX A

JUDGING TOXICITY BY BIOASSAY

From "First Progress Report of Aquatic Life Advisory Committee"
Sewage and Industrial Wastes, Vol. 27, No. 3, pp. 321-331

In the development of pollution-abatement programs it would be convenient to have precise standards established for the disposal of industrial wastes. The tasks of both industry and regulatory agencies would be much simpler if such standards could be set. Since the oxygen and pH requirements of many fishes and some other aquatic organisms have been studied intensively, it is possible to recommend criteria pertaining to these factors. Criteria for many substances, however, cannot now be established because the requirements of fishes and other aquatic organisms have not been established quantitatively. Further, such tolerance limits as have been determined for individual waste components are not always applicable to all important species in all waters and especially to situations where a mixture of chemicals is involved.

Published data on the toxicity of industrial waste components reveal that there is considerable variation in the lethal concentrations of these substances. This fact is not surprising in view of the diversity of waters that have been used as diluents and of organisms used in the experiments described in the literature. The toxicity of many substances varies greatly from one water to the next, depending on the nature and quantities of dissolved materials present. For instance, some waters are highly buffered and are able to neutralize large amounts of acids, whereas others are only slightly buffered and consequently can neutralize little acid. Ammonia is much more toxic in alkaline waters than in neutral and acid waters. On the other hand, cyanides are more toxic in acid waters than in alkaline waters.

Aquatic organisms differ greatly in their susceptibility to toxic substances. For example, fishes of the black bass and sunfish family are more resistant to copper than are trout and many species of the minnow family, whereas the water flea, *Daphnia*, may be more susceptible than any fish species.

Although tolerance limits are known for many materials, little is known of the toxicity of mixtures. Because wastes are usually mix-

tures and not single substances, the toxicity of the mixture and not the individual components must be considered. The toxicity of such mixtures cannot always be predicted on the basis of the individual toxicities of their components, as in the case where almost equally toxic solutions of copper and zinc salts are combined. The resulting mixture can be much more rapidly fatal to fish than were the component solutions.

In order that wastes may be disposed of in a manner that will leave the receiving water unimpaired biologically, evaluation of their toxicity to organisms in the receiving water is essential. This end can be achieved only through bioassays performed under appropriate experimental conditions.

Procedures for conducting bioassays for the direct evaluation of the toxicity of water pollutants have been prepared by a committee of the Federation of Sewage and Industrial Wastes Associations. The committee's report (5) contains recommendations on the choice and preparation of test animals; the selection and preparation of the experimental water; temperatures; test containers; experimental procedure, reporting, interpreting and use of bioassay results; auxiliary methods; etc. In this report it is recommended that the results of toxicity tests be expressed as median tolerance limits (TL_m). TL_m is the concentration of the substance or waste in question which kills one-half the animals in a specific period of exposure (for example, 48-hr. TL_m). These procedures are now in use in industrial, state, and federal laboratories.

Permissible concentrations of toxicants in waters receiving industrial wastes are those which can be tolerated indefinitely by all individuals (and not only 50 per cent) of all significant species of aquatic organisms, including organisms which serve in the food chains as well as fish and others of direct economic and recreational importance, in all stages of their life history. Therefore, the concentration of toxic industrial wastes should never be more than a small fraction of that concentration which under experimental conditions is demonstrably fatal within a limited period of time to 50 per cent of the test animals used in the bioassay. It is believed that the result of the 48-hr. TL_m multiplied by an application factor of 0.1 represents a concentration of waste which usually will not produce adverse effects on the aquatic organisms as a whole. In some instances more thorough investigations of the toxicity may reveal that a greater application factor is adequate. However, in other instances a much smaller application factor may be found necessary. Different application factors should be established for different types of wastes depending on their chemical and toxicological properties and the nature of the receiving water. The

application factor of 0.1 is indicative of the probable average and, therefore, is tentative and should be modified as conditions demand.

In view of these considerations the Aquatic Life Advisory Committee makes the following recommendations for controlling the disposal of toxic wastes:

1. The toxicity of wastes to aquatic life in the receiving water can most effectively and reliably be determined by means of bioassays.
2. The bioassay methods for the evaluation of acute toxicity of industrial wastes to fish described and recommended by the Committee on Research, Sub-committee on Toxicity, Section III, Federation of Sewage and Industrial Wastes Associations are endorsed.
3. The final concentration of any waste in the receiving water should be no greater than the 48-hr. $TL_m \times 0.1$ (that is, the 48-hr. TL_m as determined by the bioassay methods recommended multiplied by 0.1, the tentative application factor). Other application factors may be used in specific cases, but only after thorough investigations justifying their use have been made.

The Aquatic Life Advisory Committee, in recommending the bioassay method as a practical and applicable method for measuring the toxicity of wastes to aquatic organisms in waters receiving such wastes, recognizes that there are certain limitations to the application of the method which definitely affect interpretation of the results. Consequently, the person or persons attempting to apply bioassays to the problem must use considerable judgment in organizing each test, such as the selection of test animals, etc. Considerable care must be used in the drawing of conclusions to recognize limiting factors in each test. Therefore the committee recommends that bioassays be conducted by persons of recognized experience and training in this field. This recommendation is made for the sole purpose of assuring that the results obtained from bioassay tests be of the highest quality and greatest reliability.

APPENDIX B

SELECTED BIBLIOGRAPHIES

In general, bioassay will be conducted by personnel well qualified to carry out this type of investigation and who will be familiar with pertinent literature. For those who must set up organizations and appraise the reports of bioassays at administrative levels, the following selected list of literature will aid in developing background in the problems and requirements of this technique. The bibliography on biological indicators of pollution has been selected with the same objective that applies to literature of bioassays and is not intended to be comprehensive and complete for the biologists.

BIOASSAYS AND TOXICITY

- Belding, D. L., Toxicity Experiments with Fish in Reference to Trade Waste Pollution. *Trans. Amer. Fisheries Soc.*, 57,: 100-119 (1927).
- Burdick, G. E., and Lipschuetz, M., Toxicity of Ferro- and Ferricyanide Solutions to Fish, and Determination of the Cause of Mortality. *Trans. Amer. Fisheries Soc.*, 78,: 192-202 (1950).
- Carpenter, K. E., The Lethal Action of Soluble Metallic Salts on Fishes. *Brit. Jour. Exp. Biol.*, 4,: 378-390 (1927).
- Carpenter, K. E., Further Researches on the Action of Metallic Salts on Fishes. *Jour. Exper. Zool.*, 56,: 407-422 (1930).
- Chipman, W. A. and Galtsoff, P. S., Effects of Oil Mixed with Carbonized Sand on Aquatic Animals. *Special Scientific Report—Fisheries No. 1*, Fish and Wildlife Service,: 1-52 Appendix 1, figs. 1-25 (1949).
- Collier, A., and Ray, S. M., An Automatic Proportioning Apparatus for Experimental Study of the Effects of Chemical Solutions on Aquatic Animals. *Science*, 107,: 576-577 (1948).
- Daugherty, F. M., Jr., A proposed Toxicity Test for Industrial Wastes to be Discharged to Marine Waters. *Sewage and Indus. Wastes*, 23,: 1029-1031 (1951).
- Doudoroff, P., and Katz, M., Critical Review of Literature on the Toxicity of Industrial Wastes and Their Components to Fish. I. Alkalies, Acids, and Inorganic Gases. *Sewage and Indus. Wastes*, 22,: 1432-1458 (1950).
- Doudoroff, P., et al., Bio-Assay Methods for the Evaluation of Acute Toxicity of Industrial Wastes to Fish. *Sewage and Indus. Wastes*, 23,: 1380-1397 (1951).
- Doudoroff, P., and Katz, M., Critical Review of Literature on the Toxicity of Industrial Wastes and Their Components to Fish. II. The Metals, as Salts. *Sewage and Indus. Wastes*, 25,: 802-839 (1953).
- Edminster, J. O., and Gray, J. W., The Toxicity Threshold for Three Chlorides and Three Acids to the Fry of the Whitefish (*Coregonus clupeaformis*) and Yellow Pickerel (*Stizostedion v. vitreum*). *Prog. Fish-Culturist*, 10,: 105-106 (1948).

- Ellis, M. M., Detection and Measurement of Stream Pollution. U. S. Bur. of Fisheries; *Bull. Bur. Fisheries*, 48,: 365-437 (1937).
- Ellis, M. M., Westfall, B. A. and Ellis, M. D., Determination of Water Quality. *Research Report 9*, U. S. Fish and Wildlife Service, 22 pp. (1946).
- Galtsoff, P. S., Reaction of Oysters to Chlorination. *Research Report 11*, Fish and Wildlife Service, 28 pp. (1946).
- Guelyard, F., and Duval, M., Toxicite comparee de divers acides pour les poissons (epinoches). *Compt. rend. Acad. Sci.*, 175,:1243-1245 (1922).
- Grindley, J., Toxicity to Rainbow Trout and Minnows of Some Substances Known to be Present in Waste Water Discharged to Rivers. *Ann. Appl. Biol.*, 33,: 103-112 (1946).
- Hart, W. B., Doudoroff, P., and Greenbank, J., The Evaluation of the Toxicity of Industrial Wastes, Chemicals, and Other Substances to Fresh-Water Fishes. The Atlantic Refining Co., Philadelphia, Pa., 317 pp. (1945).
- Hasler, Arthur D. and Wisby, Warren J., Use of Fish for the Olfactory Assay of Pollutants (phenols) in Water. *Trans. Amer. Fisheries. Soc.* 79,: 64-70 (1950).
- Herbert, D. W. M., Measurement of the Toxicity of Substances to Fish. Institute of Sewage Purification, London, Eng. 8 pp. (1952).
- Jones, J. R. E., The Toxic Action of Heavy Metal Salts on the Three-Spined Stickleback (*Gasterosteus aculeatus*). *Jour. Exp. Biol.*, 12,: 165-173 (1935).
- Jones, J. R. E., The Relative Toxicity of Salts of Lead, Zinc, and Copper to the Stickleback (*Gasterosteus aculeatus* L.) and the Effect of Calcium on the Toxicity of Lead and Zinc Salts. *Jour. Exp. Biol.*, 15,: 394-407 (1938).
- Jones, J. R. E., The Relation Between the Electrolytic Solution Pressures of the Metals and Their Toxicity to the Stickleback (*Gasterosteus aculeatus* L.). *Jour. Exp. Biol.*, 16,: 425-457 (1939).
- Jones, J. R. E., The Toxicity of Double Chlorides of Mercury and Sodium. I. Experiments with the Minnow, *Phoxinus phoxinus* (L.). *Jour. Exp. Biol.*, 17,: 325-330 (1940).
- Jones, J. R. E., A Study of the Zinc Polluted River Ystwyth in North Cardiganshire, Wales. *Ann. Appl. Biol.*, 27,: 368-378 (1940).
- Jones, J. R. E., The Reactions of *Pygosteus pungitius* L. to Toxic Solutions. *Jour. Exp. Biol.*, 24,: 110-122 (1947).
- Jones, J. R. E., A Further Study of the Reactions of Fish to Toxic Solutions. *Jour. Exp. Biol.*, 25,: 22-34 (1948).
- Karsten, A., Investigation of the Effect of Cyanide on Black Hills Trout. *Black Hills Eng.*, 22: 145-174 (1954).
- Klassen, C. W., Hasfurther, W. A., and Young, M. K., The Toxicity of Hexavalent Chromium to Sunfish and Bluegills. *Proc. Fourth Industrial Waste Conf.*, Purdue Univ.: 229-237 (1949).
- Mackereth, F. J., and Smyly, W. J. P., Toxicity of Copper in Solution to the Stone-Loach. *Nature* 168,: 1130 (1951).
- Powers, E. B., The Goldfish (*Carassius carassius*) as a Test Animal in the Study of Toxicity. *Ill. Biol. Monographs*, 4, 127-193 (1917).
- Shelford, V. E., An Experimental Study of the Effects of Gas Waste upon Fishes, with Especial Reference to Stream Pollution. *Bull. Ill. State Lab. Nat. Hist.*, 11,: 381-412 (1917).
- Shelford, V. E., Ways and Means of Measuring the Dangers of Pollution to Fisheries. *Bull. Ill. State Nat. Hist. Surv.*, 13: 25-42 (1918).
- Trama, F. B., The Acute Toxicity of Phenol to the Common Bluegill (*Lepomis macrochirus* Rafinesque). *Notulae Naturae* of the Academy of Natural Sciences of Philadelphia. No. 269,: 1-10. (1955).
- Van Horn, W. M., Anderson, J. B., and Katz, M., The Effect of Kraft Pulp Mill Wastes on Some Aquatic Organisms. *Trans. Amer. Fisheries Soc.*, 79: 55-63 (1950).

BIOLOGICAL INDICATORS OF POLLUTION

- Bartsch, A. F., Biological Aspects of Stream Pollution. *Sewage Works Jour.*, 20,: 292-302 (1948).
- Bartsch, A. F. and Churchill, Warren S., Biotic response to stream pollution during artificial aeration. Limnological Aspects of Water Supply and Waste Disposal. Am. Assoc. for Adv. Sci., pp. 33-48 (1949).
- Beck, Wm. M., Jr., Studies in Stream Pollution Biology. I. A Simplified Ecological Classification of Organisms. *Quart. Jour. Fla. Acad. Sci.*, 17: 211-227 (1954).
- Brinley, F. J., Biological Ohio River Pollution Survey. I. Biological Zones in a Polluted Stream. II. Plankton Algae as an Indicator of the Sanitary Condition of a Stream. *Sewage Works Jour.*, 14,: 147-159 (1942).
- Butcher, R. W., The Biological Detection of Pollution. Inst. of Sewage Purification. Paper presented at a meeting of the Midland Branch, Birmingham, Eng., pp. 3-8, (1946).
- Claassen, P. W., The Biology of Stream Pollution. *Sewage Works Jour.*, 4,: 165-172 (1932).
- Farrell, M. A., A Biological Survey of the St. Lawrence Watershed. II. Studies of the Bottom Fauna in Polluted Areas. *Biol. Sur. No. 5, Suppl. Twentieth Ann. Rept., State of New York, Conservation Dept.*, pp. 192-197 (1930).
- Fjordingstad, E., The microphyte communities of two stagnant fresh-water ditches rich in H_2S . *Dansk Bot. Arkiv.* 14(2),: 1-44 (1950).
- Fjordingstad, E., The microflora of the river Molleae, with special reference to the relation of benthic algae to pollution. *Folia Limnologica Scandinavica* 5,: 1-123 (1950).
- Forbes, S. A., and Richardson, R. E., Studies on the Biology of the Upper Illinois River. *Bull. Ill. State Lab. of Nat. Hist.*, 9,: Art. 10: 481-574 (1913).
- Forbes, S. A., and Richardson, R. E., Some Recent Changes in Illinois River Biology. *Bull. Ill. Nat. Hist. Sur.*, 13, Art. 6: 141-156 (1919).
- Gaufin, A. R., and Tarzwell, C. M., Aquatic Invertebrates as Indicators of Stream Pollution. *Pub. Health Reports*, 67,: 57-64 (1952).
- Gaufin, A. R., and Tarzwell, C. M., Environmental Changes in a Polluted Stream During Winter. *The Amer. Midland Naturalist*, 54,: 78-88 (July 1955).
- Henderson, C., Value of the Bottom Sampler in Demonstrating the Effects of Pollution on Fish-Food Organisms and Fish in the Shenandoah River. *Prog. Fish-Cult.*, 11,: 217-230 (1949).
- Huet, Marcel, La pollution des eaux. L'analyse biologique des eaux polluées. *Sta. Rech. Groenendaal (Belgium)*, Serie D. No. 9,: 1-31 (1949).
- Katz, M., and Gaufin, A. R., The Effects of Sewage Pollution on the Fish Population of a Midwestern Stream. *Trans. Am. Fish Soc.*, 82,: 156-165 (1953).
- Kehr, R. W., *et al.* Study of the Pollution and Natural Purification of the Scioto River. *Pub. Health Bull. No. 276*,: 153 pp (1941).
- Kolkwitz, R., and Marsson, M., Oekologie der pflanzlichen Saprobien. *Ber. d. Deut. Bot. Gessell.* XXVIa,: 505-519 (1908).
- Kolkwitz, R., and Marsson, M., Oekologie der tierischen Saprobien. *Int. Rev. d. ges. Hydrobiologie u. Hydrographie* 2,: 126-152 (1909).
- Kolkwitz, R., Biologie des Trinkwassers, Abwassers und der Vorfluter. *Rubner, Gruber and Ficker's Handbuch der Hygiene.* 2, abt. 2: 335-386 Leipzig: S. Hirzel. (1911).
- Lackey, James B., Stream Microbiology, Stream Sanitation by Earle B. Phelps. John Wiley and Sons, New York, Chapter 7: 227-265 (1944).
- Lackey, James B., Protozoan plankton as indicators of pollution in a flowing stream. *Pub. Health Rep.* 53,: (46): 2037-2058 (1938).
- Patrick, Ruth, Biological Measure of Stream Conditions. *Sewage and Indust. Wastes*, 22,: 926-938 (1950).

- Purdy, W. C., Study of the Pollution and Natural Purification of the Ohio River. Part I. The Plankton and Related Organisms. *Pub. Health Bull. No. 104*,: 130-191 (1916).
- Purdy, W. C., Study of the Pollution and Natural Purification of the Ohio River. Part I. The Plankton and Related Organisms. *Pub. Health Bull. No. 131*,: 78 pp (1922).
- Purdy, W. C., Biology of Polluted Waters. *Jour. Am. Water Wks. Assn.*, 16,: 45-54 (1926).
- Purdy, W. C., Activities of Plankton in the Natural Purification of Polluted Water. *Jour. Am. Pub. Health Assn.*, 18,: 468-475 (1928).
- Purdy, W. C., A study of the pollution and natural purification of the Illinois River. II. The plankton and related organisms. *Pub. Health Bull. No. 198*,: 211 pp. (1930).
- Richardson, R. E., Changes in the Bottom and Shore Fauna of the Middle Illinois River and Its Connecting Lakes Since 1913-1915 as a Result of the Increased Southward of Sewage Pollution. *Bull. Ill. Nat. Hist. Sur.*, 14, Art. 4: 33-75 (1921).
- Richardson, R. E., Changes in the Small Bottom Fauna of Peoria Lake, 1920 to 1922. *Bull. Ill. Nat. Hist. Survey*, 15, Art. 5: 327-388 (1925).
- Richardson, R. E., The Bottom Fauna of the Middle Illinois River 1913-1923. Its Distribution Abundance Valuation and Index Value in the Study of Stream Pollution. *Bull. Ill. Nat. Hist. Sur.*, 17, Art. 12: 387-475 (1928).
- Tarzwel, C. M., and Doudoroff, P., Applications of Biological Research for the Control of Industrial Wastes. *Proc. Nat. Tech. Task Comm. on Ind. Wastes*, Cincinnati, Ohio, June 3-4, 1952: 1-18 (1952).
- Tarzwel, C. M., and Gaufin, A. R., Some Important Biological Effects of Pollution Often Disregarded in Stream Surveys. *Proc. of the Eighth Industrial Waste Conf.*, Purdue Univ. 38 pp. (1953).
- Van Horn, W. M., The Biological Indices of Stream Quality. *Proc. Fifth Ind. Waste Conf.*, Purdue Univ.: 215-222 (1949).
- Weston, R. S., and Turner, C. E., Studies on the Digestion of a Sewage Filter Effluent by a Small and Otherwise Unpolluted Stream. *Mass. Inst. Tech. Sanitary Research Lab. and Sewage Exp. Sta.*, 10,: 1-43 (1917).
- Whipple, G. C., Fair, G. M., and Whipple, M. C., The Microscopy of Drinking Water. 585 pp. John Wiley and Sons, Inc. (1948).

For further information regarding water-pollution control activities in a specific state signatory to the Ohio River Valley Water Sanitation Compact, inquiries may be directed to:

ILLINOIS	Technical Secretary State Sanitary Water Board Springfield, Illinois
INDIANA	Technical Secretary Indiana Stream Pollution Control Board 1330 West Michigan Street Indianapolis 7, Indiana
KENTUCKY	Executive Director Kentucky Water Pollution Control Commission 620 South Third Street Louisville 1, Kentucky
NEW YORK	Executive Secretary New York State Water Pollution Control Board New York State Dept. of Health Albany 1, New York
OHIO	Chief Engineer Division of Sanitary Engineering Ohio Department of Health 306 Ohio Departments Building Columbus 15, Ohio
PENNSYLVANIA	Sanitary Water Board Box No. 90 Harrisburg, Pennsylvania
VIRGINIA	Executive Secretary State Water Control Board 415 West Franklin Street Richmond 20, Virginia
WEST VIRGINIA	Executive Secretary State Water Commission 1709 Washington Street, East Charleston, West Virginia