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# Aquatic Toxicology: An Evolving Science

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## *Introduction*

Man has historically discharged his wastes into streams and because the United States has many large streams – some of them swift-flowing – disposal of large volumes of wastes may not have much apparent effect. This practice has changed in the past 20–25 years because public pressure has demanded, via legislation and programs, that the deterioration of water supplies cease and that upgrading programs be carried out. As a part of the efforts, much emphasis has been placed on the development, utility and standardization of toxicity tests using aquatic species. Thus, the scientific discipline of 'aquatic toxicology' has developed into a small but recognized branch of science. Although it is not possible to provide every detail of the field for this conference, we plan to consider some salient events that brought the discipline into recognition, some basic differences between aquatic and mammalian toxicology, and some directions in which aquatic toxicologists appear to be heading.

## *Brief History of Aquatic Toxicology*

The first report in which an aquatic species was involved in testing appears to be that of Beudant, who in 1816 conducted experiments in which he subjected 15 species of freshwater mollusks to salt solutions [*Anderson*, 1980]. Later, he subjected 38 marine mollusk species to fresh-water and dilutions of seawater. His interest did not concern pollution but the physiological mechanisms of adaptation. Nevertheless, his methods were similar to those used today in toxicity testing.

In the late 1800s, the aim of research became ensuring that domestic water supplies were safe for drinking, and, as a result, most of the effort was directed toward practical aspects of the study of algae and other microscopic aquatic organisms – particularly organisms responsible for diseases. Emphasis was on the influence of organic enrichment on plankton populations and the effects of increased organism growth in water supplies, especially those responsible for changes in taste, odor, turbidity, and color [*Tarzwell*, 1978].

Although it is difficult to document the first time fish served as test organisms, the establishment of the American Fisheries Society in 1870 and the US Commission of Fish and Fisheries in the early 1870s indicated an awareness of elimination or reduction of fish resources in many streams receiving sewage or industrial wastes. In 1885, McDonald reported on his studies of the toxic effects of wastes from ammoniacal works on young shad [*Tarzwell*, 1978]. This work was the beginning of 60 years of what later became known as 'pickle-jar-bioassays' because the investigators used large-mouthed glass pickle jars as test containers. The jars were given away free (difficult to beat the price), were easily cleaned, were nontoxic because of the purity of the glass, and had large mouths for efficient gaseous exchange. During the decades of these efforts, though the tests were short term, test solutions not renewed (static), and methods nonstandardized, large volumes of excellent data and conclusions resulted from them. *Ellis* [1937] perhaps prepared one of the best studies, and certainly the most comprehensive, on environmental requirements of aquatic species. He also published a paper on 'Detection and measurement of stream pollution', which is a classic. His recommendation of a minimum of 5.0 mg/l oxygen for a viable fish population in the field is still used and referenced.

The need for focusing attention to common objectives and methodologies in the 1950s resulted in the First Seminar on Biological Problems in Water Pollution held in Cincinnati in 1956; another was held later, in 1959 [*Tarzwell*, 1978]. At the first seminar, biological indicators of pollution, water quality criteria, use and value of bioassays were discussed. The second emphasized the various effects of pesticides on aquatic life and the practical aspects of biological findings regarding pollution abatement. A third Seminar on Biological Problems occurred in 1962 just after passage of legislation providing for construction of the two national water quality laboratories at Duluth, Minn., and Narragansett, R.I. This last seminar aroused the interest of many scientists for aquatic testing and laid the foundation for the involvement in the environmental movement.

Modern day aquatic toxicology owes its popularity to Rachael Carson and her book, *Silent Spring*. Following its publication, the American public began educating itself about effects of chemicals on the environment. The environmental awareness movement peaked during the late 1960s and early 1970s and, although the movement appears to be less intense today, an appreciation for the delicate balance of terrestrial and aquatic ecosystems continues.

The first annual meeting of the Aquatic Toxicology Section of the American Society of Testing and Materials (ASTM) took place in 1977. On several occasions since then more than 600 have registered at the annual meeting. In November a similar number of aquatic toxicologists attend the annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC). Smaller groups concerned with water quality meet regularly as part of the American Fisheries Society, the Society of Toxicology, and others. A journal entitled 'Aquatic Toxicology' is presently published by Elsevier Biomedical Press and perhaps 20–25 others also place emphasis on water quality or pollution-related subjects.

### *Legislation Establishing Aquatic Toxicology as a Viable Discipline*

Although the establishment of aquatic toxicology as a viable science parallels the environmental movement, legislation chartered its direction. Legislation that recommends, addresses, or requires aquatic testing includes: Federal Insecticide, Fungicide & Rodenticide Act (FIFRA); Toxic Substances Control Act (TSCA); Water Pollution Control Act (Clean Water Act); Marine Protection Research and Sanctuaries Act (MPRSA); Resource Conservation and Recovery Act (RCRA); and National Environmental Policy Act (NEPA).

The sequence of the legislation is related to the development of guidelines for testing. FIFRA is first because most of the research, test procedures, and test organisms were required in testing prior to registration of pesticides. Most of the procedures in the interim guidelines for TSCA were simply adopted from FIFRA; likewise, procedures for the Clean Water Act and the Marine Protection Research and Sanctuaries Act (Section 103), though fewer, are similar to FIFRA's. The use of aquatic tests for implementing RCRA is minimal at present, with little or no aquatic testing required for NEPA.

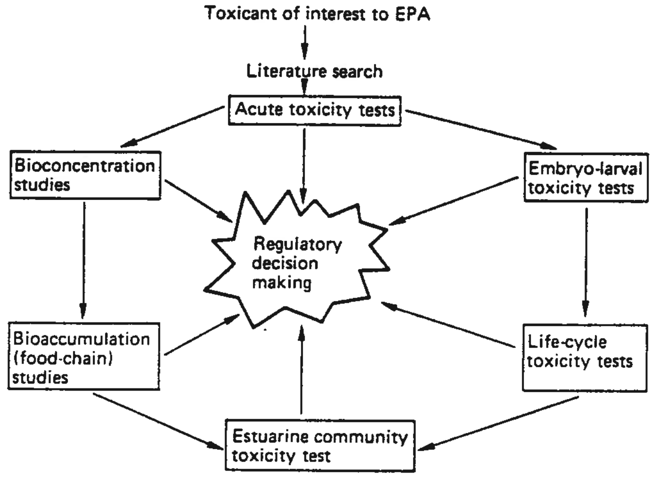


*Fig. 1.* Organization of investigational divisions at the Environmental Research Laboratory, Gulf Breeze, Fla. [source: personal commun., *Thomas W. Duke*].

As a consequence of legislation and the need for guidelines, protocols and quality assurance plans, government and private laboratories are important centers for research today. As an example, figure 1 outlines the program at Environmental Protection Agency's (EPA) Environmental Research Laboratory, Gulf Breeze, Fla. The areas of research reflect efforts in support of legislation affecting the registration of pesticides or the industrial use of synthetic organics. In many respects, this program is similar to those in private laboratories where the 'standard' tests are conducted. Figure 2 shows a slightly different perspective in research programs because the interest of this laboratory (National Fisheries Research Laboratory, Columbia, Mo.) is not in response to legislation but concerns the chemicals or practices used in the management of fishery resources. Perhaps a bit more effort is directed towards so-called 'basic' research and parallels laboratories associated with universities. At present, several laboratories with capabilities of aquatic toxicology are operated by the Department of Interior, National Marine Fisheries Service and EPA. Several major chemical manufacturers have established toxicology laboratories, with Proctor & Gamble probably being the first to build. DuPont, Kodak, Dow Chemical, Monsanto, Eli Lilly, and Allied Chemical have opened 'in-house' testing facilities. Although I do not have an accurate count, I

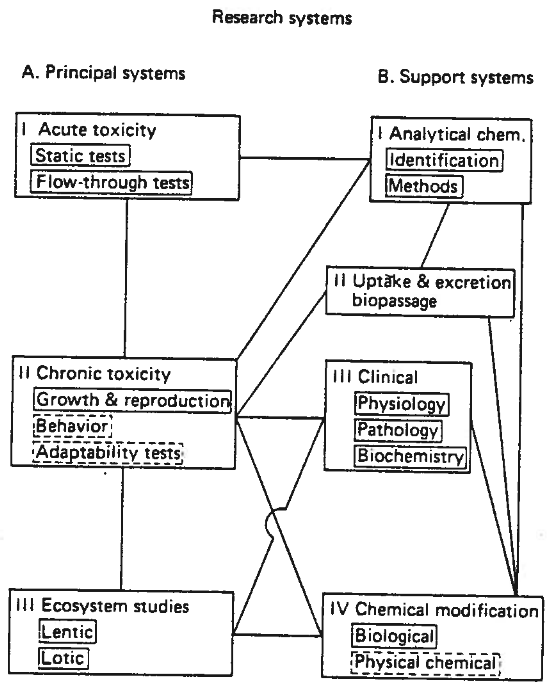


*Fig. 2.* Organization of investigational divisions at the Fish-Pesticide Research Laboratory (National Fisheries Research Laboratory), Columbia, Mo. [source: *Schoettger*, 1978].

know of at least 25 private testing facilities – some associated with universities as part of a department or a research foundation.

As a brief digression, I want to mention that, despite current economic woes, that public's desire for strong environmental standards for water has not diminished. Recently, *Humphrey Taylor*, President of Lou Harris and Associates, told a Clean Water conference that the 79% of persons concerned with curbing *water* pollution ranks favorably with the percent concerned with economic problems, and is higher than those concerned with *air* pollution [Inside EPA, vol. 3, No. 22, June 4, 1982]. According to *Taylor*, 93% of the population is against easing the Clean Water Act. Of those favoring the Act, 52% want it stricter and 41% are satisfied with the present law. 4% want it less strict.

On toxics, *Taylor* said 65% of those sampled thought pollution of lakes and rivers by toxics was 'very serious' as opposed to 22% who thought the burning of coal and polluting the air was 'very serious'. The five most serious toxic problems, according to the results of the polls, were: (1) toxic pollution of rivers and lakes; (2) leakage from hazardous waste; (3) nontoxic pollutants; (4) disposal of hazardous waste, and (5) pollution of drinking water.

### *Basic Differences between Mammalian and Aquatic Toxicology*

Mammalian and aquatic toxicology are definitely governed by dissimilar objectives, experimental methods, and limitations (table I). Mammalian toxicologists are usually interested in protecting one species – man. The test species used are small-to-medium-sized mammals considered to be fairly close analogues for man. Aquatic toxicologists, on the other hand, are concerned with the tens of thousands of species from rooted plants and plankton to fishes, birds or mammals that consume them. The goal is to understand what the environmental consequences of pollution are and how whole ecological communities (including terrestrial food chains) can be protected from particular substances. Aquatic toxicologists usually study fish and invertebrates to protect the integrity of their communities; however, more and more often a need arises to address the potential of substances to move through aquatic food chains that reach man.

Aquatic toxicologists perform studies that vary over a wide range of temperatures, salinities, and substrates, from totally marine environments to the freshwater environments, including the alpine lakes of the Rocky Mountains. The intermixing of saltwater and freshwater provides a unique biological environment: the estuary. The estuary serves a vital function in the life histories of many aquatic species; for example, the commercially-important penaeid shrimp of the South Atlantic and Gulf Coast reproduce in the open ocean, then migrate to the estuaries where they achieve their maximum growth before returning back to the ocean to complete their life cycle.

As a result of the wide range of habitats and various roles of organisms in ecosystems, about two dozen species are commonly used in aquatic toxicity tests (table II). Because of the long history of research on freshwater organisms, the technology of producing commonly-used freshwater test species is relatively advanced. Nevertheless, research on marine species suitable for testing is fast closing the gap. Generally, the most desirable species for toxicity testing are those that mature quickly, have relatively

*Table I.* Mammalian and aquatic toxicology differ in many respects [source: *Dagani*, 1980]

| Mammalian toxicology | Aquatic toxicology |
|---|---|
| Objective: to protect humans | Objective: to protect populations of many diverse species |
| Must almost always rely on animal models since experimentation on humans is unethical | Can experiment directly on species of concern |
| Species of interest (man) is known; thus, degree of extrapolation is certain | Not able to identify and test all species of concern; thus, degree of extrapolation is uncertain |
| Test organisms are warm-blooded (body temperature is relatively uniform and nearly independent of environmental temperature); thus, toxicity is rather predictable | Test organisms live in a variable environment and are cold-blooded (body temperature is variable and usually dependent on environmental temperature); thus, toxicity may not be sufficiently predictable |
| Margin for error is significant as the result of error is socially unacceptable | Margin for error can be lower since social implications are less severe |
| The dose of a test chemical usually can be measured directly and accurately, and administered by a number of routes | The 'dose' is known only in terms of the chemical's concentration in water and the length of exposure to it; the actual 'absorbed dose' is sometimes determined experimentally using bioconcentration and metabolism studies |
| Extensive 'basic' research has been conducted; emphasis has been on understanding mechanisms of toxic action | Hardly any 'basic' research has been conducted; emphasis has been on measuring toxic effects, with an eye toward regulatory needs |
| Test methods are well developed, their usefulness and limits well understood | Test methods are relatively new, their usefulness uncertain |

short life cycles, thrive in laboratory aquaria and are easily handled, relatively disease-free, and small as adults.

Among the commonly used freshwater organisms are rainbow trout, bluegill sunfish, fathead minnow, and the crustacean *Daphnia magna;* saltwater organisms include oysters, penaeid shrimp, mysids, spot and sheepshead minnows.

*Table II.* About two dozen diverse species are commonly used in aquatic toxicity tests [source: *Dagani*, 1980]

| | Freshwater species | Saltwater species |
|---|---|---|
| Vertebrates | bluegill sunfish, *Lepomis macrochirus* | sheepshead minnow, *Cyprinodon variegatus* |
| | rainbow trout, *Salmo gairdneri* | striped mullet, *Mugil cephalus* |
| | fathead minnow, *Pimephales promelas* | spot, *Leiostomus xanthurus* |
| | channel catfish, *Ictalurus punctatus* | mummichog, *Fundulus heteroclitus* |
| | brook trout, *Savelinus fontinalis* | |
| Invertebrates | water flea, *Daphnia magna* or *D. pulex* | shrimp, *Penaeus* sp. |
| | midge, *Chironomus* sp. | grass shrimp, *Palaemonetes vulgaris* |
| | scud, *Gammarus fasciatus* | mysid shrimp, *Mysidopsis bahia* |
| | crayfish, *Orconectes* sp. | eastern oyster, *Crassostrea virginica* |
| | grass shrimp, *Palaemonetes kadiakensis* | blue mussel, *Mytilus edulis* |
| Plants | algae, *Selenastrum* sp. | algae, *Skeletonema* sp. |
| | algae, *Navicula* sp. | algae, *Dunaliella* sp. |
| | algae, *Chlorella* sp. | algae, *Thalassiosira* sp. |
| | algae, *Microcystis* sp. | |
| | duckweed, *Lemna minor* | |

### *Evaluation of Hazard or Risk: A Developing Area in Aquatic Toxicology*

One of the areas in which aquatic toxicologists must make decisions with increasing regularity pertains to safety, risk, and hazard assessment. These factors are obviously closely allied and the decision-making processes are similar. All assessments begin by gathering facts that can form a basis for making sound scientific judgments relevant to toxicity, environmental concentrations, sources of the material – whether point- or nonpoint sources; stability of the material; and the physical, chemical and biological processes in the environment. (A good reference is the text, 'Analyzing the hazard evaluation process,' by *Dickson* et al. [1979].) The question may arise as to why aquatic toxicologists are frequently asked for evaluations, and one answer is that most have several of the following ecologically-based disciplines in their backgrounds: fishery biology; aquatic macroinvertebrate biology; population biology; geology; physiology; analytical chemistry; limnology/oceanography; aquatic ecology (field biology).

In addition, many specialists also have experience with computer programming, experimental design, electronics (I might add that ancillary experience in glass cutting, glass blowing, carpentry and plumbing are extremely helpful). In the last few years more often than not mammalian, terrestrial and aquatic toxicologists compare the environmental chemistry and fate data with the biological effects data in all these realms and then define the extent of testing required for a valid assessment procedure.

### *Field Studies: The Ultimate Toxicity Test*

Last, one of the questions frequently addressed by aquatic as opposed to mammalian toxicologists is what happens when materials enter the aquatic environment. Three types of field studies come to mind. The first is referred to as a model ecosystem or microcosm [*Metcalf and Lu*, 1973]. Although it has been conducted outside in small ponds, the procedure usually incorporates a series of substrate and water mixtures along with 2–6 species of organisms. After a brief time for system equilibration (the amount of time depends on the size and complexity of the microcosm), the substance of interest is introduced and, in theory, environmental rates of transport, volatilization, degradation, bioconcentration, sediment/water partitioning, etc., are all determined from one test.

The second type of field study is the so-called 'field validation' study. At present, some involve pesticides – many of them with new chemical structure or mechanisms of action. Examples are pheromones, chitin inhibitors, etc. Some of the effects on supposedly nontarget species cannot be predicted from laboratory structure/functional studies. The only method at present is to evaluate them by testing the substance in the environment on a limited basis. Interestingly, field validation studies require the broad training in ecology of many aquatic toxicologists.

A third type of field study is commonly called 'in situ toxicity testing'. In situ studies have been a part of aquatic toxicology for several decades. In fact, as early as the 1930s, *Ellis* [1937] based many of his conclusions on 'in situ' studies using the water below industrial or municipal outfalls as test water rather than high quality laboratory water. Today, many private and state regulatory divisions have mobile trailers or vans equipped with holding tanks, temperature-controlled baths, diluter systems for conducting either static or flow-through tests using water on site. As an example of a site study, we recently determined the effects of ammonia on brown trout below the Breckenridge. Colo. municipal treatment plant. which has an outfall into the Blue River. The concern was that the volume of effluent produced by the skiing industry from December through March was maximum at the same time the trout alevins (juveniles) were migrating downstream from spawning sites.

Mobile bioassay activity will probably increase with the implementation of guidelines for deriving site-specific water quality criteria. These procedures allow states to recognize that national a criterion (for example. copper) may not be appropriate for a stream that is habitat-limited for a particular fishery or use. I suspect that the need for such capability is likely to increase toward the mid-1980s. then diminish in the late 1980s.

### *Future Consideration*

One of the obvious need in the recent past and a need for the next few years is the use of toxicity tests to monitor the progress or lack thereof of industrial or municipal wastewater treatment systems. The ultimate test for a treatment system is what happens below any outfall in terms of a 'biological' response. Does the fishery still exist? Are unwanted aquatic plants choking the waterway? Are macroinvertebrate communities still present? The only way to assess change is with a biological test tailored to the question. Furthermore, no proven procedure apparently exists for verifying that site-specific limits (criteria or standards) other than biological testing of water at the site.

Perhaps one of the most overlooked uses of aquatic species in testing is to determine the quality of ground water. According to a recent report in *Water Well Journal* [Anonymous, 1981], at least 100 million Americans depend directly on ground water for drinking. More headlines are appearing that add to citizen concern as to the quality of a critical resource we

*Table III.* Comparison of water quality criteria for several priority pollutants and 'maximum recommended concentrations[1] in drinking water

| | Protective of human health µg/l | Freshwater water quality criterion µg/l |
|---|---|---|
| Acrolein | 320.0 | 21.0 |
| Cadmium | 10.0 | 6.3[2] |
| Cyanide | 200.0 | 3.5 |
| Endosulfan | 74.0 | 0.056 |
| Endrin | 1.0 | 0.0023 |
| Lead | 50.0 | 20.0[2] |
| Mercury | 0.144 | 0.00057 |
| Nickel | 13.4 | 160.0[2] |
| Pentachlorophenol | 30.0[3] | 3.2 |
| Selenium | 10.0 | 35.0 |
| Silver | 50.0 | 13.0[2] |
| Zinc | 5.000.0[3] | 570.0[2] |

[1] Criteria (or guidance) published in Federal Register, vol. 45, No. 231, Friday, November 28, 1980.
[2] Criterion established for a water hardness of 200 mg/l.
[3] Based on organoleptic (taste and odor) of tainted fish.

take for granted. Several species – in particular the daphnids – could be 'first screen' or Tier I test organisms used in conjunction with cursory chemical analyses. Many of the hazardous materials finding their way into drinking water are organics, to which *Daphnia* are acutely sensitive. I have attempted to make this point in table III, which compares selected criteria believed to be protective of aquatic life to the drinking water standards. If ground water, for example, contained a mixture similar to those shown in the table, it would be much more cost effective to test the water with an aquatic species instead of expensive bulk chemical analysis. *Daphnia magna* can be used in literally hundreds of tests per day to test for acute toxicity, and if in depth studies were warranted on the basis of preliminary estimates of toxicity, complete chemical analysis could be conducted.

Perhaps aquatic toxicologists could play a larger role in the research on the effects of acid precipitation. Evidence of adverse ecological effects has become obvious from field data with most being circumstantial as opposed to laboratory-derived data. For instance, in the wealth of field observations from Scandinavian countries, the association between loss of algae, rooted plants, invertebrates, and fishes and increased acidity is well established. However, the ability to predict a certain threshold from acidity is much less certain. It appears that laboratory studies are one viable way to arrive at such predictions. Although several excellent studies of chronic effects of lowered pH has to be demonstrated with fishes, I am not aware of any macroinvertebrates tested in the laboratory or field under chronic stress from pH. One suggestion is that those locales, believed to be sensitive to pH change because of the poor buffering capacity of the watershed, could have several acute and chronic tests conducted 'on site' using mobile facilities. One way to predict environmental impact is by using local or known imported indicator species particularly sensitive to pH with actual site waters.

In conclusion, I want to emphasize that the goal of the aquatic toxicologist is to protect the aquatic community. Regardless of specialty or subdiscipline, the desire of the aquatic toxicologist is to preserve a few selected species that are of intense interest to the general public (e.g. game fish). We have definitely made tremendous progress in the past two decades, primarily in the laboratory. Directing more effort toward understanding 'real' communities in our natural aquatic environments is a prudent and necessary goal. The challenges posed by increased demand for water, contamination of surface waters from waste sites, and acid precipitation are certainly substantial ones for us all.

## *Summary*

During the 15-year span between 1965 and 1980, the fledgling science of 'aquatic toxicology' was coming of age. Practiced by a few biologists as an 'art' from the 1920s on, the field has grown phenomenally in the late 1960s and early 1970s as a result of legislation. Well-known environmental problems drove home the fact that many substances including synthetic organics can enter water, accumulate in organisms, and finally reach terrestrial food chains, which include man.

Although current economic conditions have curtailed growth of aquatic toxicology, independent testing is a viable emerging industry. In addition, the Environmental Protection Agency (EPA) maintains several freshwater and marine applied research and development toxicology laboratories. Also, Fish and Wildlife (Department of Interior) and National Marine Fisheries laboratories have programs similar to those of EPA. As a result of directed research in these and other private laboratories, about two dozen diverse aquatic species are now commonly used in testing. If all of these species were tested with the same substance, the results would provide a fair estimate of its effects on freshwater and marine communities.

Generally, organisms used routinely are fishes, crustaceans, and algae. Emphasis on these three groups is probably the result of: (1) a long history of research in culturing them resulting in (2) fair agreement of methodology. Acute tests are conducted for about 48–96 h, and results are expressed as $LC_{50}$ (median lethal concentration to 50% of the test organisms).

Increasingly, aquatic toxicologists are asked to address questions that 'stretch' their expertise and training. Questions of pollutant transport, chemical sorption onto or desorption from natural substances, metabolic pathways, enzyme inhibition, or modifications of tissue or cellular structure are common. In short, aquatic toxicologists are often asked to evaluate the potential for hazard to the whole environment for a particular chemical, or, more frequently, for a combination of substances. The field is becoming more specialized, with individuals who may explore the toxicity of organic-metal complexes or the effects of chitin-inhibiting pesticides on nontarget insect populations.

Perhaps the most complicated, yet important, question of all is how fish-in-a-tank data extrapolate to real-world aquatic communities and several areas where aquatic toxicology might play some critical roles are discussed. These are 'site-specific' studies, quality of ground water and acid precipitation effects.

## *References*


Anderson, B.G.: Aquatic invertebrates in tolerance investigations from Aristotle to Naumann; in Buikema, Cairns, Aquatic invertebrate bioassays, ASTM STP 715, pp. 3–35 (American Society for Testing and Materials, 1980).

Anonymous: New Jersey community decontaminates well water. Water Well J. *35:* 58–59 (1981).

Dagani, R.: Aquatic toxicology matures, gains importance. Chem. Eng. News *June 30:* 18–23 (1980).

Dickson, K.L.; Maki, A.W.; Cairns, J., Jr.: Analyzing the hazard evaluation process. Proc. Wkshop, Waterville Valley 1978, pp. 159 (American Fisheries Society, 1979).

Ellis, M.M.: Detection and measurement of stream pollution. Bull. Bureau Fisheries *48:* 365–437 (1937).

Metcalf, R.L.; Lu, P.Y.: Environmental distribution and metabolic fate of key industrial pollutants and pesticides in a model ecosystem. Report U2LU-WRC-0069, University of Illinois (Urbana Water Resources Center, 1973).

Schoettger, R.A.: Registration of pesticides: considerations in conducting aquatic toxicity tests; in Proc. 1st and 2nd USA-USSR Symp. Effects of Pollutants upon Aquatic Ecosystems, 1978, EPA-600/3-78-076, pp. 166–180.

Tarzwell, C.M.: A brief history of water pollution research in the United States; in Proc. 1st and 2nd USA-USSR Symp. Effects of Pollutants Upon Aquatic Ecosystems, 1978, EPA-600/3-78-076, pp. 11–31.


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