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BIOMONITORING

Using Living Organisms to Track Complex Pollutants
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As the list of potential pollutants becomes more lengthy and complex, regulators and scientists are turning to an ancient concept called biomonitoring to observe and protect water quality.

Biomonitoring may be a new term but it has been used for centuries. Simply put, biomonitoring uses living organisms to gauge damage that pollutants or toxic substances can or may have caused.

For example, medieval kings used a kind of biomonitor - the food taster - to determine if rivals were poisoning the evening meal. During the industrial revolution, miners took caged canaries with them as they ventured deep into the shafts. If the bird got sick or died, the miners knew it was time to get back to the surface.

Three types of biomonitoring have specific applications to today's water issues.

First, stream surveys can provide useful information on water quality by inventorying the diversity of species that are present or absent. If particularly sensitive species can't be found or if massive numbers of fish suddenly die (the fish kills on the Trinity and Pecos rivers come to mind), a pollution problem may exist.

Secondly, the Environmental Protection Agency (EPA) and the Texas Water Commission (TWC) are both implementing programs to require cities and industries to use biomonitors to test the toxicity of their wastewater discharges. In the programs, waterfleas and fathead minnows are placed in effluents for up to a week. If too many of the test organisms die or suffer mutations, expensive follow-up tests called toxicity reduction evaluations can be required to pinpoint the source of the problem and correct it.

Use of biomonitoring programs as a regulatory tool is a sensitive issue for many wastewater plant owners and operators who question its effectiveness and cost.

Third, scientists are developing tests called bioassays that use animals such as mice and rats, turtles, hydra (a type of coral), earthworms and others to determineif pollutants are causing death or mutations in test animals and to infer if similar effects may result in humans. Studies have involved rats living near hazardous waste sites, turtles native to areas near nuclear power plants and laboratory species.

The importance and use of biomonitoring may increase in the near future. The EPA estimates that roughly 550 municipal and industrial dischargers in Texas may be required to use biomonitoring to test their discharges in the near future.

In a broader sense, the concept of biomonitoring is gaining wider acceptance in both the scientific and regulatory communities, and new ways of utilizing biomonitoring are being developed. For example, scientists in Colorado are using biomonitors to assess the safety of groundwater supplies and researchers in Missouri and other states are employing freshwater mussels to measure water quality in streams.

Why is there this sudden interest in biomonitoring? Two primary reasons come to mind.

First, the whole point of controlling pollution is to minimize damage to living organisms. Biomonitoring provides a way to measure how pollutants affect the health and viability of aquatic life and other animal species. Some biomonitors, in particular bioassays, have direct implications for human health.

Secondly, there is virtually an unlimited number of potential pollutants being used in a myriad of combinations, but EPA and other regulators have only developed numerical standards for roughly 150 of them. This means that the effect of a number of contaminants can't be accounted for with conventional approaches. Even if all the pollutants could be accounted for, numeric standards couldn't detect the cumulative or synergistic effects of a number of contaminants that might be in the stream at the same time. The toxic effect of some chemicals increases dramatically when other pollutants are also present. Because biomonitoring focuses on the impacts on the test organism regardless of the source, the system lets scientists know if toxic problems may be present. Biomonitoring also identifies scant concentrations of pollutants which may not be detectable by modern methods, but which still may be toxic.

Ecological Surveys

One of the most obvious ways to utilize biomonitoring is to measure the change in the number of species in a river, lake or stream as a result of pollution or man's activities.

Ken Stewart, an aquatic entomologist with the University of North Texas specializes in studies of stonefly populations. As a group, stoneflies can't tolerate low oxygen levels, silt buildup and industrial discharges. Some species of stoneflies, however, are more tolerant than others. Stewart's approach is to collect samples of the insects from selected streams. By comparing the tolerances of different species of stoneflies in streams, the amount of water pollution can be estimated. Currently, Stewart and his colleagues are modeling streams in Arkansas to determine how water quality affects stonefly populations.

Aquatic insects have been used to monitor water pollution in rivers and streams in many studies. One recent study investigated whether a mine on the Red River near Taos, New Mexico was polluting the river with trace metals. Researchers collected water and native insects such as mayflies, stoneflies and caddisflies and then analyzed the insect tissue to see if trace metal concentrations had increased. Results suggest that levels of trace metals were greater downstream of the mine's discharges (Lynch, et al., 1988).

Another project at the University of North Texas is sampling the Trinity River in the Dallas-Fort Worth area to measure how man's activities are affecting the diversity and health of its ecosystem. The project, which is being conducted by Ken Dickson, John Rodgers and Tom Waller of the Institute of Applied Sciences, involves inventorying the number of aquatic insects and fish in the Trinity River and assessing whether water and sediments in the river are toxic to them. Follow-up analyses will involve detailed laboratory studies to assess toxicity levels with daphnia (waterfleas), midges (gnats), bluegills, fathead minnows, and algae collected from the river system.

Although biomonitoring is utilized almost exclusively for surface water, researchers at Colorado State University (Ward, et al., 1989) have proposed that it may also be useful for assessing groundwater quality. Their research focused on measuring populations of small aquatic worms, crustaceans and insects in riverine aquifer systems. Comparing the number of species that are present before and after man's activity or a specific pollution event could provide clues to the amount of groundwater degradation that occurred.

Another way to use natural stream conditions for biomonitoring is to measure the amount of toxic chemicals individual species have accumulated and resulting ill health effects. Mussels and clams, for example, have been used to monitor heavy metal, organic chemical, and fecal coliform concentrations in both fresh water and salt water because they provide information on the bioavailability of contaminants and because they reflect current water quality trends. Terry Wade, a researcher in the Oceanography Department at Texas A&M, is among those analyzing pollutant levels in oysters and sediments in the Gulf of Mexico as part of the National Oceanic and Atmospheric Administration Mussel Watch Program. Samples are being taken from so sites (including many Texas locations) along the coast of the GuH of Mexico. The study is collecting baseline data on levels of coprostanol (which indicates If sewage is present), pesticides, PCBs, and other organic chemicals that can be used to measure future changes in pollution (Wade, et al., 1988). In addition, the Texas Department of Health has an ongoing program to monitor contaminants in oyster tissues. When excessive levels of pollutants are detected, those areas are closed to oyster harvesting. Freshwater species such as the pocketbook mussel have also been used to monitor tailings from lead mines in Missouri (Czamezki, 1987).

The impact of chronic (long-term) exposure of turtles to low levels of radioactive waste has been a focus of John Bickham's work at Texas A&M University. Bickham, a researcher in the Wildlife and Fisheries Sciences Department, has studied turtles from the Savannah River nuclear plant in South Carolina to see if genetic abnormalities occurred when they were exposed to low levels of radioactive waste. Because the turtles can live for up to 20 years, they provide a good measure of chronic toxicity (many laboratory studies measure only short-term or acute damage). Using a technique called flow cytometry to analyze genetic mutations that may have taken place in the cells, Bickham and his colleagues determined that changes in DNA structure and cell abnormalities had taken place in the turtles (Bickham, et al., 1988).

Even animals that wouldn't usually be associated with water can yield critical information about water quality. Bickham, Kirk Brown, Barrett Lyne of the Soil and Crop Sciences Department, and others are working to determine the amount and types of groundwater pollution at EPA Superfund sites. The studies utilized flow cytometry to determine if mice living near the site suffered DNA damage (McBee and Bickham, 1988). Because the mice were continuously exposed to the site, they represented the maximum amount of exposure an animal may get from this sort of toxic waste. Analyses revealed that the mice suffered chromosome damage.

The use of earthworms to assess the impact of pollutants from landfills, industrial wastes, and river sediments is being investigated by scientists at the University of North Texas. Lloyd Fitzpatrick, Barney Venables and Art Goven of the Biology Department are studying the impact of pollutants on the immune system of earthworms (Rodriguez-Grau, et al., 1989). A recent study involved placing night crawlers in the soil of a Superfund site to determine the effects of short-term exposure to chlordane and other chemicals. Results suggest that the earthworm immune system becomes suppressed after 48 hours and the worms lose the ability to fight off bacteria. Fitzpatrick says that earthworms are good biological sentinels because their immune systems are sufficiently similar to humans and because they live underground where wastes are concentrated.

Measuring levels of toxic chemicals in animal tissues can also help assess how much pollution has occurred in streams and rivers. The U.S. Fish and Wildlife Service has collected data on levels of PCBs and other pollutants in fish and wildlife tissue from the Trinity River (Irwin, 1988) and other regions throughout the state. A U.S. Geological Survey report (Wells, et al., 1987) measured levels of cadmium, chromium and other heavy metals in fishes, turtles, crabs and birds in the Lower Rio Grande Valley to determine the effect of irrigation drainage on water quality. EPA has developed methods that use native biological species to evaluate the impact of wastewater treatment plant discharges on water quality in rivers (EPA, 1989a).

Testing Wastewater Effluents: The Regulatory Approach

Specific biomonitoring tests are now being required by the EPA and TWC to identify pollutants in addition to those contaminants covered by numeric standards. The specific intent of the program is to protect habitat for fish and other aquatic life, not human health. However, many of the chemicals detected through biomonitoring could also harm people.

The impetus for EPA to require biomonitoring stems from Section 101 of the Clean Water Act which states that the "discharge of toxic pollutants in toxic amounts be prohibited.. EPA's national policy calls for "integrating both biological and chemical methods to address toxic and nonconventional pollutants.. At the regional level, EPA has stated that "no discharge shall result in any instream acute or chronic aquatic toxicity" and "no bioaccumulation shall result which threatens human health."

Biomonitoring regulations were first implemented in Texas in 1987. Of the nearly 550 major municipal and industrial discharges in Texas that are covered by EPA's National Point Discharge Elimination System, 50 cities including Dallas, Fort Worth, Houston and Amarillo, and 77 industries now have requirements for biomonitoring written into their permits. That number will rise dramatically in the next 18 months as permits come up for renewal and are updated to include biomonitoring. In general, cities with populations over 10,000 or with treatment plant capacities of more than one million gallons per day (MGD), and major industries that consistently have toxic substances in their wastewaters will probably have to conduct biomonitoring tests. Nationally, roughly 40% of major industries and 10% of large cities now conduct biomonitoring tests. The tests can be monthly, quarterly or semi-annually, depending on the size of the facility and if toxicity problems are expected.

In bask terms, the EPA regulations involve subjecting living organisms to wastewater effluents at concentrations that simulate low flow conditions in the mixing zone (the area where effluents merge with natural conditions in the river or stream). Detailed publications (EPA, 1988, 1989b) describe specific procedures and protocols.

The tests can be conducted by in-house staff or by outside laboratories. The regional EPA office in Dallas has a listing of labs that have performed biomonitoring tests, although there is not yet a program to license and certify labs.

Two tests are typically conducted: acute tests simulate short-term exposure and chronictests mimic longer- term effects. EPA recommends that plants discharging into fresh water utilize fathead minnows and different species of daphnia for both the acute and chronic tests. For salt water, EPA requires that mysid shrimp and sheepshead minnows be used for both tests. Individual chronic tests usually cost roughly $1,000 while acute tests average $200.

Toxicity is judged by the number of organisms that survive the tests and the impact wastewaters have on sublethal characteristics such as reproduction and growth. It should be noted that the test species may also suffer other symptoms including abnormal bodily functions and movement, birth defects, mutations, and effects on the nervous system. Calculations are made of the lethal dose that kills 50% of the test species and the highest concentration that permits propagation of fish and other aquatic IHe in receiving waters.

Problems can be relatively simple to identify and correct such as excessive amounts of chlorine or ammonia nitrogen, or can involve intricate interactions of complex chemicals.

Many university studies have investigated various aspects of biomonitoring. Tom Waller of the Biology Department at the University of North Texas has conducted numerous investigations on different species of daphnia utilized in biomonitoring tests. Research by Waller and his colleagues has included studies of the ideal diet and methods to culture the water fleas, whether different daphnia species can be utilized simultaneously in biomonitoring tests, and drying daphnia embryos so they can later be reactivated when testing would take place (Knight and Waller, 1987). Studies by Ken Dickson of the Institute of Applied Science at the University of North Texas (Parkerton, et al., 1988) have investigated whether EPA test procedures accurately reflect real conditions. The studies compared if daphnia and fathead minnows that have been acclimated to natural stream conditions were more resistant to toxicity than species reared in laboratories, and contrasted the effects of using ambient water and laboratory prepared water samples.

At Texas Christian University, biologist Kyle Hoagland and graduate student Loretta Mokry compared the acute toxicities of five pyrethroid insecticides to two daphnia species (Mokry and Hoagland, 1989). Results suggest that some newly developed pyrethroid insecticides are more toxic to daphnia than earlier pesticides.

Neal Armstrong of the Civil Engineering Department at the University of Texas at Austin teaches a short course on biomonitoring to wastewater treatment plant professionals. Some universities including Southwest Texas State University at San Marcos are establishing full-scale labs to conduct biomonitoring experiments and to perform the tests for others.

Toxicity Reduction Evaluations

If wastewaters repeatedly have toxic problems (usually three or four tests), EPA may require that a comprehensive
procedure called a toxicity reduction evaluation (TRE) be conducted. The purpose of the TRE (EPA, 1989c M is to methodically determine what specific, chemical or class of chemicals killed the species in question and to take corrective actions. TREs usually consist of three stages: identifying the toxic chemicals, developing treatment and management solutions and implementing the new treatment technology. EPA also has the power to fine those who do not carry out a TRE, or who do not meet prescribed deadlines ranging from six months to a year for small facilities and up to two years for major dischargers. One alternative to penalizing those that do not finish TREs within established timetables is being used in Colorado (Michael, 1989). The system bases fines on the diligence that discharges show in carrying out the steps of a TRE; not the speed at which results are compiled.

Preliminary Texas results show that 13 dischargers have failed at least one toxicity test. Of that number, six plants have produced multiple toxic results, and five of the six are conducting TREs. There may also be a number of Texas cities that have toxic problems that are not yet biomonitoring. EPA recently sued San Antonio, El Paso, Beaumont, Galveston, Nacogdoches, Waxahachie, Brownsville, McAllen, and Mineral Wells for illegally allowing industries to dump untreated effluents into public wastewater treatment plants.

The TRE process is often drawn out and expensive: an informal TWRI survey for this article showed that the process can often last up to two years and maycost more than $500,000.

A protocol to identify causes of toxicity in complex industrial wastewaters is being developed by Ken Dickson and others at the Institute of Applied Science at the University of North Texas (Gasith, et al., 1988). The methodology uses daphnia to measure the relative effectiveness of different treatment technologies and may help identify whether toxicity was caused by a specific class of chemicals.

TWC is also beginning to initiate its own regulations. As part of the state's new toxic pollutant policy for surface waters, the TWC will require biomonitoring for all municipal wastewater facilities with capacities greater than 5 MGD. So far, the TWC has required 35 industries and three cities to conduct regular biomonitoring tests. Biomonitoring may also be required if toxic materials are present in wastewaters on a consistent basis or if the TWC believes that the potential for a toxic problem exists. TWC regulations allow dischargers to utilize "standard" EPA test species, but species that are native to local streams or rivers can be approved on a case-by-case basis.

Reaction to the new regulations has been mixed. cities and water districts are critical of the potential high costs of carrying out biomonitoring tests and TREs. The Trinity River Authority has estimated that annual biomonitoring costs at one of its major wastewater plants could exceed $100,000 if toxic problems are present.

Other key issues that have been raised include whether EPA has the Authority to use biomonitoring as an "enforcement tool" when the program was originally intended to be an "information collection" device and whether municipal wastewater treatment plants (not industries) should be the focus of a toxic chemical program.

Bioassays

Bioassays are specific tests and procedures which can be run to test the effects of toxic substances on selected animal species. Bioassays can be carried out utilizing either native or standard laboratory animals. This section focuses on laboratory experiments (examples of bioassays that employ indigenous animals are given in the "Ecological Surveys. section of this report).

In Texas, much of the work involving bioassays is being conducted by a research center that is developing new methods to assess the toxic potential of chemical wastes in aquifers and soils near landfills and EPA Superfund sites. The center is being led by Steve Safe of the Texas ABM University Veterinary Physiology and Pharmacology

Department. Scientists from Texas A&M University, the Baylor College of Medicine, and Texas ABM University at Galveston may participate.

Safe is now developing inexpensive bioassays to gauge the impact of PCBs, dioxins, complex hydrocarbons and other pollutants on rodents, fish and other animals. The bioassays utilize whole animal *(in* vivo) responses to pollutants and cultured *(in* vitro) cells to measure specific responses. One project involved determining if results from *in* vitro studies could be applied to estimate *in vivo* affects of exposure to PCBs and other complex chemicals (Safe, 1987). Other bioassays are being developed for different contaminants. Results are promising and suggest that there is a correlation of more than 90% between results from cell culture bioassays and whole animal studies. Ultimately, the studies could quantify health risks from specific complex chemicals, isolate the most toxic constituent from a complex chemical mixture, and provide detailed descriptions of the chemical and biological effects of hazardous waste sites. Safe has also investigated levels of dioxin and other chemicals in fish from the Great Lakes.

A bioassay that combines *in vivo* studies of a coral-like animal called the hydra and *in* vitro studies of rat embryos is being developed by Tim Phillips, a researcher in the Veterinary Public Health Department at Texas ABM University. The bioassay compares the toxicity of a class of chemicals called "T-2 toxins" which occur in seeds of contaminated cereal grains and have been known to cause intestinal damage, hemorrhages, skin disease, altered heart rates and other maladies in rats and other mammals. First, the hydra are exposed to the chemicals and hazard indexes are developed for both adult animals and developing embryos. Later, the tests are confirmed using rat embryos cultured *in* vitro. The studies have identified which specific T-2 chemicals are the most toxic (Mayura, et al. 1989).

Summary

Biomonitoring, the use of living organisms to monitor the effect of pollutants, is an evolving way to identify whether complex chemicals in today's environment are causing health risks to humans and other animals. At least three advantages are immediately apparent: 1) biomonitoring may detect chemicals for which standard numeric criteria have not been developed and may describe health risks caused by a number of chemicals in combination; 2) It is able to detect toxic and sublethal effects, even when the concentrations of the chemical causing the damage are below analytical limits; and 3) Biomonitoring and bioassays may tell us about the damage contaminants cause to living organisms in terms of mortality, mutations, increased incidence of cancers, and other symptoms.

Bioassays may become useful tools to predict the effect of pollutants on living organisms, to determine the extent of mutations that have occurred, and to rank hazardous waste and Superfund sites to prioritize cleanup efforts.

Bioassays that utilize *Salmonella* and other bacteria are being developed by Kirk Brown of the Soil and Crop Sciences Department at Texas ABM University. Brown has utilized the bioassay in combination with traditional analyses to determine the mutagenic characteristics of water samples from EPA Superfund sites, to rank which sites are most contaminated, to compare the inherent ability of agricultural soils to cause mutations, and to examine health risks caused by chemicals leaching into soils and groundwater from municipal landfills (Brown and Donnelly, 1988).

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