News Release

No. 0560006-3 (USACEHR develops an Army of sentinels to learn more about chemical contamination)

By Susan Phelps

Army medical researchers at Fort Detrick are developing an army of fish, frogs, bees, and shrimp to serve as sentinels for chemical contamination in the environment. This new “army” is helping scientists obtain information about chemical contamination more efficiently and effectively and is providing scientists with new information about chemicals and how they interact with the environment and each other.

The U.S. Army Center for Environmental Health Research (USACEHR), part of the U.S. Army Medical Research and Materiel Command, began developing the various sentinels in 1981 to create screening tools for hazardous substances that are unique to the military. The basic idea behind the sentinels is that non-mammalian, lower vertebrate animals show physical signs of stress when exposed to chemical contaminants. Such warning signals often indicate negative impacts to the environment that could, in turn, affect human health.

The development of an assay, or test, involves several steps. First, USACEHR identifies existing sentinel technologies and works with the researchers to refine them for a specific military concern. As the technology is refined, USACEHR develops protocols and procedures for conducting the assay and then tests the assay to validate that it is sensitive, accurate, and

reproducible. USACEHR establishes standard methods and guidelines for the assay though such programs as the American Society for Testing and Materials (ASTM) so that others can replicate the process and use the assay. Finally, the assay is tested in the field to ensure that it can be applied to real-world situations. All of these steps are necessary for the assay to gain regulatory acceptance. According to Robert Finch, Associate Director for Research at USACEHR, "Regulatory agencies require standardized, validated assay systems before allowing data to be used for regulatory purposes. They accept these data from the assays to make more informed decisions on chemical impacts on the environment."

USACEHR has several assays that are in varying stages of development. Each assay is designed to detect a specific toxicological impact, such as respiratory, reproductive, teratogenic (birth defects), carcinogenic, and neurological effects. The assays use a variety of non-mammalian vertebrates that demonstrate sensitivity to a particular toxic environment.

- **Automated Fish Ventilatory Biomonitoring System:** This system monitors the breathing patterns of fish to determine water quality. The system uses bluegill fish that characteristically prefer to "stand still" in the water. Eight fish are placed in an individually chambered box. As water passes through the chambers, electrodes monitor the electrical impulses given off by the fish's muscles as they breathe. A sudden change in breathing rate signals a change in water quality and prompts an automated water sampler to collect a sample that helps scientists pin-point the source of contamination.
- **Frog Embryo Assay (FETAX):** Developing embryos from South African clawed frogs are exposed to an environment or chemical of concern to monitor for birth defects. As the embryos transform into tadpoles

over a 96-hour time period, scientists follow their development, growth, and survival rate to see if any abnormalities or death result from being exposed to the environment of concern.

- **Honey Bee Sentinel System:** According to Tommy Shedd, research aquatic biologist at USACEHR, “Bees are flying dust mops.” They make excellent soil, air, and water samplers because they are electrostatically charged, so dust in the air and soils becomes attached to them as they forage for food in a characteristic half mile radius of their hive. Scientists test the pollen and monitor the hive’s reproduction, growth, and productivity to see if there are any biological or behavioral changes that reflect a potential problem in the environment.
- **Rapid Toxicity Assessment Test Battery:** This assessment uses a combination of assays to test a variety of sites or areas of a site and quickly screen the environment for toxicants of concern. The series of assays uses a wide spectrum of test organisms, such as bacteria, algae, rotifers, crustaceans, killifish, tadpole shrimp, and lettuce seed. If toxicity is found in the environment, the assay helps the scientist determine which sites should be cleaned up first. Some assays use African annual killifish and tadpole shrimp whose eggs can be dried and stored in containers for up to 1 year and 14 years, respectively. Fifteen minutes before scientists are ready to conduct an assay, they simply add water to the eggs and hatch instant fish or shrimp.
- **Japanese Medaka Assay:** USACEHR is developing a carcinogen assay using Japanese medaka fish. The eggs of these fish can be made to hatch at the same time, providing fish with a uniform age. The newly hatched

fish are exposed to the environment of concern and monitored to see if and when they develop cancer in their 9-month life span.

The frog embryo assay and the fish ventilatory system are the two most developed assays that are ready to be applied to a variety of situations. Aberdeen Proving Ground, for example, has built a permanent fish ventilatory system in its groundwater treatment plant. The plant treats contaminated groundwater flowing from a disposal site that contains different types of degrading munitions. The fish are used to monitor the water quality at the end of the treatment process to ensure that the facility is operating cleanly and efficiently at all times. The Maryland installation also used the frog embryo assay to ensure that contaminated groundwater flowing into a nearby stream was not a threat to human or environmental health. The National Institute of Environmental Health Sciences is using the frog embryo assay to try and determine the cause of malformed frogs in Minnesota lakes and ponds.

The sentinel systems are an added tool to a hazardous assessment that make the process more efficient, more effective, and less costly. Up until recently, scientists took quarterly air, water, and soil samples in the field, sent them to a lab to be analyzed, and waited for weeks, sometimes months, to learn the results. Through the sentinel systems, scientists are able to set up permanent facilities or a temporary network of specially designed mobile trailer labs that enable them to continuously monitor the environment of concern and detect potential problems as they are developing.

The continuous data stream and monitoring are helping scientists learn more about chemicals and how they interact with the environment. Scientist can only project the potential threat of a limited number of chemicals under specific conditions. These assays allow scientists to respond to a complex mixture of chemicals in an integrated fashion. According to Col. David

Danley, Director for Program Development at USACEHR, “We know what the effects of some specific chemicals are, but we don’t know what their affect is when they are mixed with each other and the environment. These sentinel assays give us clues to what those integrated effects are.”

The assays are also providing a more effective method for determining the appropriate amount of cleanup efforts necessary for a contaminated site. “Unlike traditional toxicity tests that involve a lot of speculation and remediation goals that are often impractical, these technologies give the next level of assurance. They add known information to the hazardous assessment that help determine exactly how toxic an environment is and what level of treatment needs to be done to support a healthy ecosystem,” said Shedd.

The assay’s data can result in significant cost savings to the cleanup of contaminated sites. Kenneth Stachiw, Director of the Environmental Conservation and Restoration Division at Aberdeen Proving Ground, estimated that the use of USACEHR fish ventilatory system at the post’s groundwater-contaminated site resulted in a cost savings/cost avoidance of $4 million to $5 million. The use of the technology also speeded up the Record of Decision process and eliminated the requirement to conduct costly studies to assure the safety of the treatment facility’s effluent. In addition, the public could understand the relationship between fish health and environmental health and so more readily accepted the treatment facility’s results.

USACEHR wants to expand the use of the assays to other military facilities and applications. In FY98, Aberdeen Proving Ground plans to use the honey bee assay to help determine if its phytoremediation project, which uses hybrid poplar trees to naturally pump and treat contaminated groundwater, is transpiring contaminants into the air. In the meantime, USACEHR continues to refine and upgrade the Army’s latest troop of soldiers in the fight against

chemical contamination. “The signals are out there in the environment,” said Shedd. “We just need to use these methodologies to understand what those signals are and what they are telling us about hazards in the environment.”

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