

BIOMONITORING METHODS FOR DRINKING WATER PROTECTION

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ABSTRACT

Two biomonitoring approaches are being developed to rapidly identify toxicity associated with toxic industrial chemicals in Army drinking water supplies. An aquatic biomonitor continuously monitors water at fixed facilities such as water treatment plants by identifying changes in fish ventilatory and movement patterns. The biomonitor responds within an hour to most chemicals at acutely toxic concentrations. In field testing at two water treatment facilities (Fort Detrick, MD and New York City), the biomonitor has been on-line at least 94% of the time and has identified toxic events at each site. The aquatic biomonitor is being transitioned to a commercial partner and will be available for use at Army facilities in FY05. To apply the biomonitoring approach to Army field water situations, an Environmental Sentinel Biomonitor (ESB) system is being developed. An Integrated Product Team (IPT) of Army users identified ESB system use scenarios and downselection criteria for cell-based toxicity sensors. Using a formal decision analysis approach, an expert panel applied the criteria to 38 technologies, recommending 14 for further testing. These toxicity sensors are now being tested against a set of 15 chemicals. The best sensor (or set of sensors) will undergo further development as part of the ESB system, which is scheduled for completion in FY08.

1. INTRODUCTION

Providing drinking water to deployed troops can utilize a large fraction of available transportation assets. Although decentralized water production could reduce the transportation burden, it will be difficult to ensure that water produced in many diverse locations is safe to drink in view of the many toxic industrial and agricultural contaminants that may be present in water and the limited number of such chemicals that can be identified rapidly in the field. Instead of relying upon chemical by chemical analysis, a biomonitoring approach uses the responses of living systems (cells, tissues, or whole organisms) to rapidly indicate the overall toxicity of the water being tested. Two biomonitoring techniques are being developed at the U.S. Army Center for Environmental Health Research (USACEHR) to help evaluate the potability of drinking water. To provide continuous, real-

time monitoring for toxicity in drinking water supplies, an aquatic biomonitor was developed for use in rear areas and at fixed Army facilities such as water treatment plants. To decrease the size, weight, and logistic requirements of the aquatic biomonitor to a level more suitable for field applications, the development of an ESB system was initiated in FY04 with support from an Army Science and Technology Objective (STO). The ESB system will utilize cell-based toxicity sensors to provide drinking water protection for a range of Army applications.

2. METHODS

2.1 Aquatic Biomonitor

The USACEHR aquatic biomonitor continuously monitors the breathing patterns of fish exposed to the water of interest. The aquatic biomonitor detects toxicity by monitoring changes in the ventilatory and movement patterns of the bluegill (*Lepomis macrochirus*). Eight fish are held in individual chambers under flow-through conditions and continuous light (to minimize daily variations in ventilatory patterns). Electrical signals generated by muscle movements of individual fish are monitored by carbon block electrodes suspended above and below each fish. The electrical signals are amplified, filtered, and passed onto a personal computer for analysis. Each input channel is independently amplified by a high gain true differential-input instrumentation amplifier; signal inputs of 0.05-1 mV are amplified by a factor of 1000. Signal interference by frequencies above 10 Hz is attenuated by low-pass filters. A secondary stage of digital amplification by a factor of 10 is also performed by the computer. Ventilatory parameters are measured, including ventilatory rate, ventilatory depth (mean signal height), gill purge (cough) frequency, and whole body movement (rapid irregular electrical signals). Each parameter is calculated at 15 s intervals, and any interval in which whole body movement was detected was excluded from calculation of the other three parameters. The 15 s intervals are summed to create a 15 min data record. Specific algorithms are described elsewhere (Shedd et al., 2002). Other test methods are similar to those described in van der Schalie, et al. (2001).

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In addition to fish ventilatory data, pH, temperature, dissolved oxygen, and conductivity are monitored every 15 min using a commercially-available water quality multiprobe. These data help to determine whether fish responses are due to the presence of toxicants or to non-toxic water quality variations.

Two statistical approaches to evaluating biomonitor data have been used. In early laboratory and field tests, data collected from each fish during a pre-exposure (baseline) period is used to establish normal limits for each fish and ventilatory parameter (van der Schalie, et al., 2001). If, during the subsequent exposure period, an individual fish parameter becomes statistically different from its normal (baseline) response, the response is said to be "out of control." A biomonitor alarm occurs when more than 70% of fish alarm during the same 15 min interval.

More recently, a neural net expert system has been used to analyze both fish behavioral patterns and basic water quality information (temperature, dissolved oxygen, pH, and conductivity) to determine when a group of fish exhibits abnormal behavior (Wroblewski, 2004). The neural network was trained using data from hundreds of bluegills monitored under laboratory and field conditions. For every 15 min monitoring interval, a toxicity index value is generated for each fish. If an individual fish has a toxicity index value of greater than one, it is detected as a novel event. If more than 70% of exposed fish (i.e., six or more of eight fish) exhibit a toxicity value greater than one, an alarm response is generated. In most cases, when the same biomonitor data are analyzed by both methods, the expert system responds as or more rapidly than the earlier baseline comparison approach.

The toxicant concentration required to elicit a rapid biomonitor response (within an hour) was evaluated in laboratory tests with chemicals having different modes of toxic action. Detailed methodologies for laboratory tests are described in van der Schalie et al. (In Press).

Field tests were conducted to determine the utility of the aquatic biomonitor for source water protection at water treatment facilities at Fort Detrick, MD and New York City. Biomonitor operation at the field sites is similar to the laboratory tests, except that new groups of fish are used about every three weeks. Monitoring continuity is maintained because each biomonitor unit has two sets of fish chambers, allowing a new set of eight fish to be acclimating in one chamber while the on-line set of fish is completing its monitoring period. When a biomonitor alarm occurs during the field tests, the biomonitor computer automatically calls specific personnel and turns on an automated refrigerated water sampler so that follow-on chemical analyses of potentially contaminated water can be conducted.

Because fish are very sensitive to residual chlorine, the biomonitor cannot be used in water distribution systems without first dechlorinating the water. As part of our development of the biomonitor system, beginning in May 2004, we evaluated a commercially-available portable dechlorination unit (Geo-Centers, Inc.) in conjunction with one of the two sets of eight fish in the biomonitor at the Fort Detrick water treatment plant; the other set of eight fish was exposed to un-chlorinated river water that was the source water for the treatment plant. Fortunately, both sets of fish (exposed to source water and product water) were in use during a toxicant event that began in May 2004.

2.2 ESB System

In the first year of the ESB system STO, an IPT of Army users was formed to identify ESB system use scenarios and toxicity sensor technology downselection criteria. After an initial survey identified 38 potential toxicity sensor technologies, an expert panel applied the criteria in the initial downselection process to select toxicity sensors for further evaluation. The downselection process followed a formal decision analysis approach (ECBC DAT, 2004).

3. RESULTS AND DISCUSSION

3.1 Aquatic Biomonitor

3.1.1 Laboratory Data

Laboratory data demonstrate the capability of the aquatic biomonitor to rapidly respond to chemicals (Table 1). As might be expected, chemicals most acutely toxic to bluegills elicit the most rapid response. A comparison to available short-term military exposure guidelines (MEGs) for water shows that, in most cases, the biomonitor responds at about the MEG levels, indicating that a biomonitor alarm thought to be toxicant-related requires further investigation and appropriate follow-up. Other studies have shown that the biomonitor responds within an hour or less to the majority of chemicals at acutely toxic levels (i.e., levels at or above the concentration lethal to 50% of exposed fish after 96 hours) (van der Schalie et al., In Press). The aquatic biomonitor appears to respond more rapidly to chemicals causing membrane irritation, narcosis, or polar narcosis than to acetylcholinesterase inhibitors or oxidative phosphorylation uncouplers (van der Schalie, et al., In Press). Although the biomonitor responds rapidly to many chemicals at concentrations of concern to humans, there are some materials to which it is insensitive (e.g., thallium and sodium fluoroacetate, Table 1). Nevertheless, the biomonitor is valuable as an investigative tool that provides continuous water

Table 1. Toxicant Concentrations Required to Elicit a Biomonitor Response in an Hour or Less.

One Hour Response Level ¹ (mg/L)	Chemical	Mode of Acute Toxic Action ²	MEG ³ (mg/L)
0.01 to 0.1	Brevetoxin	Neurotoxin	NA ⁴
	Cyanide	Cellular respiration inhibitor	6
> 0.1 to 1.0	Copper	Direct gill effects	0.42 (1 yr)
	Residual chlorine	Direct gill effects	NA
	Mercury (inorganic)	Direct gill effects	0.003
	Metham sodium	Acetylcholinesterase inhibitor	NA
	Phosdrin	Acetylcholinesterase inhibitor	NA
	Zinc	Direct gill effects	8
> 1.0 to 10	Aldicarb	Acetylcholinesterase inhibitor	0.01
	Carbaryl	Acetylcholinesterase inhibitor	1.4
	p-Chlorophenol	Polar narcosis	0.8 (ortho-)
	Dichlorvos	Acetylcholinesterase inhibitor	NA
	Malathion	Acetylcholinesterase inhibitor	0.3
	Nicotine	Central nervous system seizure agent	0.4
	Pentachlorophenol	Oxidative phosphorylation inhibitor	1.4
	Strychnine	Central nervous system seizure agent	NA
	Tetrachloroethane	Narcosis	3
	Tetrachloroethylene	Narcosis	2.8
	Ammonia		30
>10 to 100	Arsenic	Oxidative phosphorylation inhibitor (in part)	0.3
	Butyl carbitol acetate		NA
	Chloroform	Narcosis	6
	Phenol	Polar narcosis	8
	Tricaine methane sulfonate	Polar narcosis	NA
> 100	Acetone	Narcosis	NA
	Meparfynol	Narcosis	NA
	2,4-Pentanedione	Electrophile	NA
	Sodium fluoroacetate	Metabolic interference	NA
	Thallium sulfate		0.01

Data sources: van der Schalie, et al. (1979); Capute (1980); Carlson (1990); van der Schalie et al. (In Press).

¹ Minimum concentration required to elicit a biomonitor response

² Principal mode of action for acute toxicity in fish

³ Military Exposure Guideline for water (5L/day consumption, < 7 day exposure period) (USACHPPM, 2004)

⁴ NA – Not Available

monitoring and facilitates follow-up actions in response to events of concern in a water supply.

3.1.2 Field Data

The two field test sites provided contrasting situation in which biomonitor field performance could be evaluated. The Fort Detrick water treatment plant draws about 4 million liters per day of source water from the Monocacy River, a river in a watershed dominated by agricultural land use. Monocacy River water has high hardness (annual average 100 mg/L as CaCO₃), with relatively high variability and rapid changes in water quality parameters such as turbidity. The biomonitor at New York City was located on a reservoir that provided part of the 4 billion liters per day used by the city. The water is low in hardness

(annual average 22 mg/L as CaCO₃) and turbidity and has lower variability, with more gradual changes in water quality.

The biomonitor at the Fort Detrick water treatment plant has been in continuous operation at the site since October 2001. Operational data are provided for the nine month period (January to September 2004) when the expert system was in use. During this period, the biomonitor was operational 98% of the time, with 2% downtime. Routine downtime (0.5%) is primarily related to the putting a new set of fish on line (about every three weeks), while unscheduled downtime (1.5%) was related to system crashes (which have been corrected and eliminated), water quality probe related difficulties, and accidental operator shutdowns. Biomonitor

alarms occurred occasionally throughout the monitoring period; most were caused by either water quality probe malfunctions or sudden changes in water temperature (more than 1°C per hour). Software optimization now underway will lessen the number of alarms related to changes in water quality. In particular, it will be easier to retrain the expert system to accept fish responses to normal, site specific changes in water quality so that an alarm is not generated.

Since October 2001, there has been only one toxicant-related alarm at the Fort Detrick facility. The event is shown in Figure 1. When the alarm initially occurred, the biomonitor notified appropriate authorities using an auto-dialer and initiated water sampling with an automated refrigerated sample. Biomonitor alarms continued over several days, with fish mortality occurring as well (7 of 8 biomonitor fish and all fish in a holding tank).

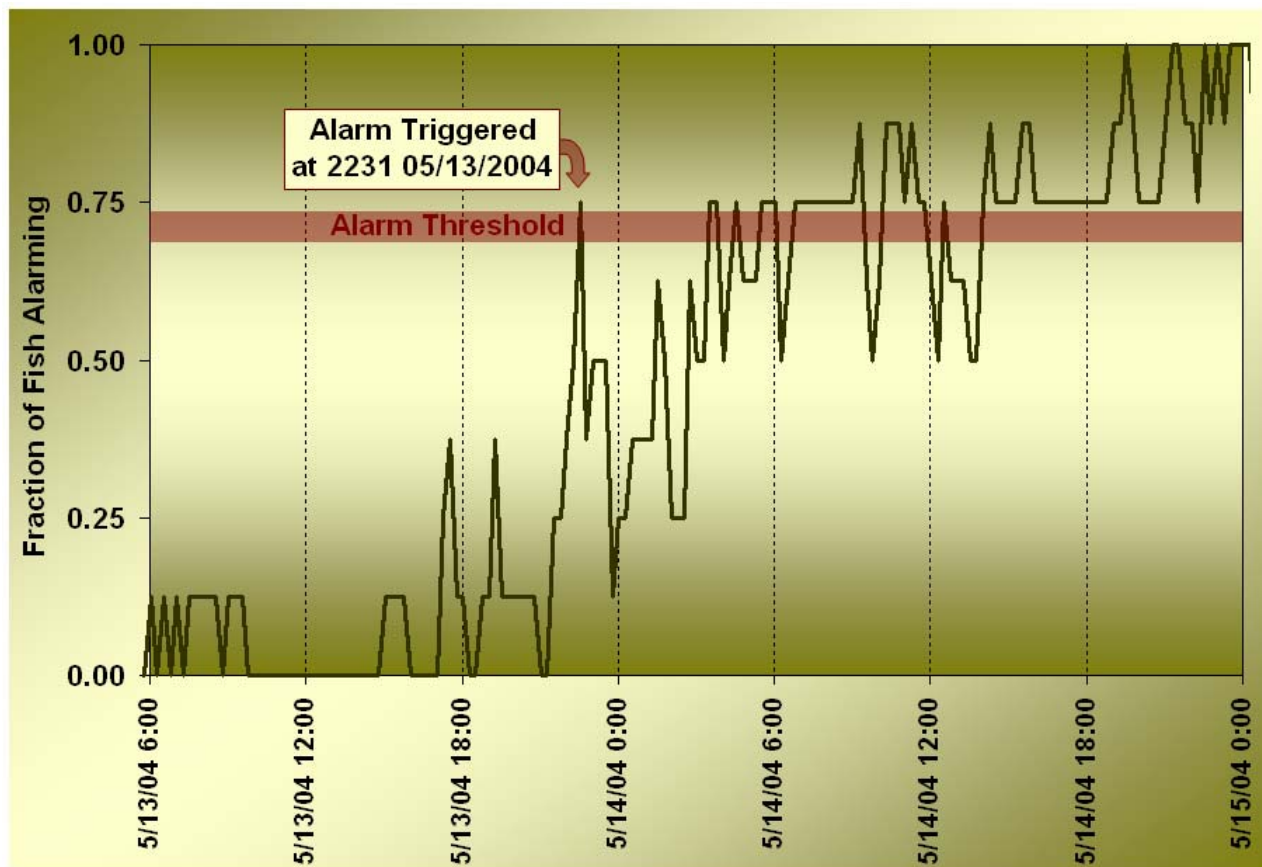


Figure 1. Aquatic biomonitor response at the Fort Detrick water treatment plant, May 2004. An alarm is triggered when 6 or more of the 8 fish monitored respond simultaneously during any one 15 min interval. The series of alarm events shown may be related to herbicide application within the Monocacy River watershed.

A key element to successful biomonitor application at any site is appropriate response to an alarm. The first step should be an investigation to confirm the likelihood of toxicity rather than equipment failure or changes in non-toxic water quality parameters. Adequate time was available for an investigation at Fort Detrick, since it takes about 48 hours for water taken from the Monocacy River to reach users at the installation. After the Fort Detrick

alarms began, remote evaluation of the data (using PC Anywhere® software) showed no obvious malfunctions or water quality variations that would explain fish behavior. Fish responses included increased ventilation and cough rates; increased cough rates are not usually associated with normal water quality variations (U.S. EPA, 2001).

Water samples from the autosampler were used for chemical analysis and confirmatory toxicity testing. A screening analysis of several samples using gas chromatography and mass spectrometry revealed two chemicals: the solvent butyl carbitol acetate and the herbicide metolachlor, suggesting that herbicide formulations used in the Monocacy River watershed may be associated with the toxicity event. However, although bluegills in the biomonitor died during the response events, no dead fish were observed in the Monocacy River, and exposure of the sensitive aquatic invertebrate *Daphnia magna* to water samples did not cause toxicity.

The set of eight biomonitor fish at the Fort Detrick water treatment plant exposed to dechlorinated Fort Detrick product water did not alarm at any time during the toxicity event in the raw river water. This demonstrated that observed source water toxicity did not persist through the water treatment process and helped Fort Detrick Garrison personnel decide not to close down the Fort Detrick water intake as a result of the biomonitor alarm.

Experiences with biomonitor operation at the New York City site were similar to Fort Detrick, although there were site to site differences in water quality composition and variation patterns (Yves Mikol, personal communication). The New York City biomonitor was operational 96% of the time over a two-year period. Routine downtime (1%) was due to test initiation and data archiving, while unscheduled downtime (3%) was due to communication failures, system crashes, or water quality multiprobe maintenance. Non-toxicant related biomonitor alarms were rare and were due to temperature fluctuations and drift in fish signal patterns through time. Fish signal pattern drift was an occasional problem with the baseline-based statistical approach, but is not a problem with the expert system currently in use.

One toxicant-related alarm occurred during the two-year biomonitoring period at New York City. At the time of the alarm, the reservoir being monitored was off-line to the city water supply, as a sediment control project was underway on the reservoir. A biomonitor alarm occurred on a Saturday morning during Memorial Day weekend in 2003. Remote examination of the data showed no evidence of equipment failure or water quality variations that might explain the alarm, and fish showed increased cough rates, suggesting possible toxicity. Subsequent investigation at the reservoir revealed an oil sheen on the water; a small amount of oil had leaked from a barge that was supporting the sediment control operation. Analysis of water taken by the automated

sampler triggered by the biomonitor alarm confirmed the presence of 47 µg/L of diesel oil.

3.1.3 Biomonitor Applications and Future Plans

Based on experiences to date with the aquatic biomonitor, Army-relevant applications include:

- Source water monitoring. As at Fort Detrick, military installations using surface waters (rivers, reservoirs, etc.) as source waters for drinking water should consider the use of a biomonitoring system for rapid identification of potential toxicity.
- Distribution water monitoring. Chlorinated product water can be used in the biomonitor if first dechlorinated, and we have demonstrated the applicability of a commercially-available portable dechlorination unit for this purpose.
- Effluent monitoring. A biomonitor has been used for several years at Aberdeen Proving Ground, where it is monitoring treated groundwater from a hazardous waste site before discharge into a Chesapeake Bay tributary (Shedd et al., 2001). The biomonitor is useful for identifying transient toxic events.
- Watershed monitoring. With funding from the Department of Defense Legacy Program, an in-stream version of the biomonitor is being integrated with water quality monitoring platforms to provide real-time watershed-wide biomonitoring capabilities. Enhanced biomonitoring capabilities can greatly improve non-point pollution monitoring at Army installations and aid in the development of sound strategies to address environmental regulations. Further benefits include the ability to identify the origin of toxic events to allow the initiation of remediation or to demonstrate that military operations are not the origin of the toxicity.

At this time, aquatic biomonitor development has been completed. The biomonitor is being transitioned to a commercial partner (Intelligent Automation Corporation) to ensure its availability to Army users (and others).

3.2 The ESB System

The IPT identified four scenarios for ESB system use: by individual soldiers (e.g., Special Forces); for testing water produced in Future Combat System (FCS) manned ground vehicles; for use in conjunction with field water production by technology such as the Tactical Water Purification System (TWPS); and at fixed facilities such as water treatment plants. The expert panel evaluated 38 technologies for use in an ESB system, including sensors utilizing a wide range of biological systems (enzyme systems, bacteria, algae, vertebrate and mammalian cells) and endpoints (luminescence, electrical activity, metabolic byproducts). Using the IPT's performance criteria, the expert panel selected 13 toxicity sensors for further testing. To facilitate comparisons of the toxicity response characteristics of these sensors, each will be tested against a set of 15 chemicals endorsed by the IPT and expert panel. In this screening process, a sensor should respond rapidly to chemical concentrations that exceed the applicable drinking water benchmark concentration, specifically the short-term (7-14 day) MEG level for that chemical. Once toxicity sensor testing is completed, a further technology downselection will be conducted by the STO expert panel, using the toxicity response data and other criteria established by the STO IPT, such as ease of use, reliability, and logistic requirements. The best toxicity sensor (or set of sensors) will undergo further development as part of the ESB system. A prototype ESB system is scheduled for completion in FY08.

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CONCLUSIONS

An automated biomonitor has been developed to provide continuous monitoring and rapid identification of toxicity in drinking water supplies (source water and product water after dechlorination). Laboratory tests show that the biomonitor will respond within an hour to a wide range of chemicals at acutely toxic concentrations. Testing at two diverse sites (Fort Detrick, MD and New York City) has demonstrated the utility of the system and its ability to respond to toxic events under field conditions. A formal technology downselection process is being utilized to identify the best toxicity sensor components for an ESB system that will provide rapid toxicity screening for several Army use scenarios.

REFERENCES

- Capute, A.J., Jr. 1980: Effect of Carbon Tetrachloride, Chloroform, and Tetrachloroethylene on the Respiratory Activity in Bluegill Sunfish (*Lepomis macrochirus*), Master's Thesis, University of Cincinnati, Cincinnati, OH.
- Carlson, R.W., 1990: Ventilatory Patterns of Bluegill (*Lepomis macrochirus*) Exposed to Organic Chemicals with Different Mechanisms of Toxic Action, *Comp. Biochem. Physiol.*, **95C**, 181-196.
- Edgewood Chemical and Biological Center Decision Analysis Team (ECBC DAT), 2004: Initial Technology Assessment for the Environmental Sentinel Biomonitor (ESB) System, U.S. Army Edgewood Chemical and Biological Center Decision Analysis Team, Aberdeen Proving Ground, Maryland.
- Kovacs, G.T.A., 2003: Electronic Sensors with Living Cellular Components, *Proc. IEEE*, **91**, 915-929.
- National Research Council (NRC), 1996: Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, DC.
- Pancrazio, J., Whelan, J.P., Borkholder, D.A., Ma, A., and Stenger, D.A., 1999: Development and Application of Cell-Based Biosensors, *Annals Biomed. Eng.*, **27**, 697-711.
- Shedd T.R., van der Schalie, W.H., Widder, M.W., Burton, D.T., and Burrows, E.P., 2001: Long-Term Operation of an Automated Fish Biomonitoring System for Continuous Effluent Acute Toxicity Surveillance. *Bull. Environ. Contam. Toxicol.*, **66**, 392-399.

- Shedd, T.R., Widder, M.W., Leach, J.D., van der Schalie, W.H., and Bishoff, R.C., 2002: Apparatus and Method for Automated Biomonitoring of Water Quality, U.S. Patent 6,393,899 B1, May 28, 2002.
- U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), 2003: Chemical Exposure Guidelines for Deployed Military Personnel, USACHPPM Technical Guide 230, Version 1.3 – Updated May 2003 with January 2004 Addendum, USACHPPM, Aberdeen Proving Ground, MD.
- U.S. Environmental Protection Agency (EPA), 2001: Real-Time Monitoring for Toxicity Caused by Harmful Algal Blooms and Other Water Quality Perturbations, EPA/600/R-01/103, National Center for Environmental Assessment - Washington Office, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.
- van der Schalie, W.H., Dickson, K.L., Westlake, G.F., Cairns, J., Jr., 1979: Fish Bioassay Monitoring of Waste Effluents. *Environ Manag.*, **3**, 217-235.
- van der Schalie, W.H., Shedd, T.R., Knechtges, P.L., and Widder, M.W., 2001: Using Higher Organisms in Biological Early Warning Systems for Real-Time Toxicity Detection, *Biosens. Bioelectron.*, **16**, 457-465.
- van der Schalie, W.H., Shedd, T.R., Widder, M.W., and Brennan, L.M.: In Press, Response Characteristics of an Aquatic Biomonitor Used for Rapid Toxicity Detection, *J. Appl. Toxicol.*
- Wroblewski, D., 2004: Expert System for Real Time Assessment of Environmental Toxicity, Phase II, Intelligent Automation Corporation, Poway, CA, U.S. Army Medical Research and Materiel Command Contract DAMD17-01-C-0011.