Online Toxicity Monitors (OTM) for Distribution System Water Quality Monitoring

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The mention of trade names or commercial products in this paper does not constitute endorsement or recommendation for use by the authors, or by their respective employers. The trade names have been included to accurately represent the equipment used for the purpose of testing and evaluation.

ABSTRACT

Drinking water distribution systems in the U.S. are vulnerable to episodic contamination events (both unintentional and intentional). The U.S. Environmental Protection Agency (EPA) is conducting research to investigate the use of broad-spectrum online toxicity monitors (OTMs) in distribution systems. This research was conducted at the Early Warning Systems Laboratory (EWSL) located at the EPA's Test and Evaluation (T&E) Facility in Cincinnati, OH and is part of ongoing OTM research for source water monitoring and watershed management. The data collected by the OTMs provides water quality managers with continuous, time-relevant information regarding water quality status. For the purposes of this testing, a pilot-scale distribution system simulator (DSS) available at the EPA T&E Facility collocated with the EWSL in Cincinnati, Ohio was used. Contaminants were injected into the distribution system and a sample line from the distribution system was connected to two OTMs to monitor their responses. Specifically, this paper presents the responses of luminescence bacteria *Vibrio fischeri* (as contained in the microLAN Toxcontrol system) and fish *Pimephales promelas* (as contained in the bbe Moldaenke ToxProtect system) to two toxic contaminants: sodium fluoroacetate and potassium cyanide under field conditions.

INTRODUCTION

Drinking water distribution systems in the U.S. (a critical and interdependent component of the nation's infrastructure) are vulnerable to both intentional and accidental episodic contamination. The EPA has developed a Response Protocol Toolbox (RPTB) to address the complex challenges of water utility's planning and response to the threat and act of intentional contamination of drinking water (Magnuson et al, 2005; U.S. EPA, 2004). One step in the contamination threat management process is to understand various warning signs that might indicate that contamination of water has occurred. Unusual water quality may serve as a warning of potential contamination if data are available. The U.S. EPA has initiated a program to investigate how changes in water quality parameters can be detected by using commercial off-the-shelf physico-chemical water quality sensors that measure the water quality in real or near real-time (Hall et al, 2007). The available physico-chemical sensors utilize general water quality parameters, such as free chlorine, oxidation reduction potential (ORP), total organic carbon (TOC), turbidity, pH,

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dissolved oxygen, specific conductance, chloride, ammonia, nitrate to detect the contamination. Generally, one or more of these water quality parameters will change due to the injection of a contaminant. However, no single chemical sensor responds to all possible contaminants nor can they give any indication of the potential toxicity of complex mixtures.

Historically, monitoring of drinking water quality has generally relied on the collection of spot (popularly known as "grab sampling") water samples followed by extraction and laboratory-based instrumental analysis for both inorganic and organic pollutants. This approach provides a snapshot of the concentrations of analyzed chemicals at a single point in time and space, again giving little indication of the samples toxicity due to single or multiple contaminants. However, research during the last two decades has shown that considerable limitations are associated with spot sampling approaches for determining total pollutant concentration (Allan et al, 2007). To overcome the limitations of grab sampling techniques, online monitors are a topic of much interest for water security applications; although there is a significant level of debate regarding their effectiveness as part of contamination warning system (ISLI, 1999). A variety of online chemical and biological instruments (popularly known as biomonitors, here referred to as on-line toxicity monitors) are being tested for use in distribution system water quality modeling applications.

OTMs are based on the toxicological response of an organism to a contaminant or mixture of contaminant (Allan et al, 2007; Baldwin & Kramer, 1991). An acute toxicity measurement based on physiological or behavioral changes is used to provide a rapid warning in response to deterioration of water quality (Allan et al, 2007; Baldwin & Kramer, 1991). A number of organisms that include fish species, daphnia, algae and bacteria have been used for biomonitoring (Allan, et al, 2007; Gerhardt et al, 2005; Baldwin et al, 1994). These online continuous systems provide a rapid evaluation and detection of temporal variation in water quality and toxicity that can not be achieved through standard approaches to chemical monitoring (Allan et al, 2007). The EPA's EWSL in Cincinnati, Ohio has been conducting research to develop and test biological online toxicity monitors for deployment in water distribution system and watershed management to obtain time-relevant information regarding the status of water quality. This paper presents the responses of luminescence bacteria *Vibrio fischeri* as contained in MicroLAN Toxcontrol and fathead minnows (*Pimephales promelas)* as contained in the bbe-Moldaenke ToxProtect system to potassium cyanide and sodium fluoroacetate.

METHODS AND MATERIALS

OTM#1: MicroLAN Toxcontrol System

The MicroLAN Toxcontrol system uses the luminescence values of the bioassay (*Vibrio fischeri)* in reference and sample water to evaluate the responses and express it as percent inhibition. The software creates an alarm if the inhibition exceeds 10%. A typical 30 minutes measurement cycle consists of 2.5 minutes for preparation of bioassay suspension, 5 minutes for temperature adjustment, 2.5 minutes for test sample

preparation, 15 minutes measurement time, 2.5 minutes for rinsing and 3 minutes break time. Direct comparison of luminescence values in reference and sample water are shown at 1 minute interval during the 15 minutes measurement time and the summary of mean inhibition and correction factor are shown at the end of each measurement cycle. A correction factor indicates the status of the bioassay by comparing the luminescence values in reference water at the beginning and end of the measurement cycle. The bioassay was prepared from freeze dried *Vibrio fischeri* (NRRL B-11177) using media prepared following procedures outlined in ISO 11348-1:1998(E). A 2% sodium chloride solution with 5mg/L sodium thiosulfate was used as a diluent to provide the bioassay a marine environment and to de-chlorinate the sample water respectively. Baseline tests demonstrated no noticeable inhibitions of the *Vibrio fischeri* due to addition of sodium thiosulfate at the selected concentration.

OTM#2: bbe-Moldaenke ToxProtect System
The bbe-Moldaenke ToxProtect system uses the movement of fish to evaluate the toxicity level of water. The movements are detected by a matrix of light emitting diode (LED) light barriers in the instrument. The toxicity alarm level is calculated using both the number of light barriers broken and their location within the matrix per unit time. The swimming pattern or movement of the fish in the instrument test chamber changes due to toxicant exposure causing the changes in toxicity alarm level. The activity level, specific activity and top covered LED are parameters that determine the toxicity alarm level. This level will increase to yellow warning level or red alarm level if the activity drops below 4.0 imp/fish/min or the specific activity or top covered LED increases above 15.0 and 0.50% respectively. The alarm level is indicated by a green, yellow or red light signal on the instrument.

The fathead minnows are held under continuous light for a minimum of 2 weeks before being transferred to the instrument test chamber with an inlet water flow of 120 to 135 l/h. A sodium thiosulfate solution is pumped into the inlet water before reaching the instrument test chamber to remove chlorine. The fish are monitored for a baseline period of 24 hours pre- and post-exposure.

DSS and Injection of Contaminants
A pilot scale DSS was used for conducting the toxicant exposure experiments. The DSS consists of 3 inch-diameter, 1,250 feet-long, glass-lined cast iron pipe. During the testing, the DSS was operated at a flow rate of 5 gpm. Contaminants were injected into the DSS using a peristaltic pump. The stock concentration and the pump speed were adjusted to achieve the desired concentration of the contaminants. Sample water from the DSS was supplied to the instruments through a sampling port, located approximately 80 ft from the point of injection. Previous flow-tracing studies determined the toxicant reaches the ToxProtect and Toxcontrol in the EWSL within 7 minutes from start of injection at the 5 gpm DSS operational rate.

Experimental Test Runs
Sodium fluoroacetate and potassium cyanide were used as toxic contaminants for evaluating the responses of the OTMs. Sodium fluoroacetate tests were conducted at 1.0

and 10.0 ppm concentration. Potassium cyanide tests were conducted at 1.0, 0.1 and 0.01 ppm concentrations. Duplicate test runs were conducted for each concentration for both contaminants. The exposure time for one of the1.0 ppm (nominal) fluoroacetate experiment was 20 minutes and all other experiments were conducted for a 90 minute exposure period.

RESULTS AND DISCUSSIONS

MicroLAN Toxcontrol System Response

The exposure concentration of the contaminant in the Toxcontrol system was 50% of the DSS concentration due to mixing of diluent and sample in the instrument. The 1.0 and 10.0 ppm sodium fluoroacetate provides 0.5 and 5.0 ppm exposure concentrations respectively in the Toxcontrol system. The 1.0, 0.1 and 0.01 ppm cyanide provides 0.5, 0.05 and 0.005 ppm exposure concentrations respectively in the Toxcontrol system. The responses recorded by the Toxcontrol during 0.5 and 5.0 (nominal) sodium fluoroacetate are shown in Figures 1 & 2 respectively. No significant increases in inhibitions were observed during exposure in comparison with that during pre- and post-exposure periods. Further investigation of this lack of instrument response revealed that this observation is consistent with that reported by Zurita et al (2007) that *Vibrio fischeri* is less sensitive to sodium fluoroacetate. Zurita et al (2007) research indicates that although sodium fluoroacetate blocks the citric acid cycle of the kreb cycle, it does not interfere with other major energy producing activities during metabolism. Figure 3 shows an example of variation of correction factors to represent the status of the *Vibrio fischeri* culture during exposure. The correction factors during exposure were within the recommended range of 0.6 – 1.3. Duplicate tests were conducted for these two concentrations and similar inhibitions and correction factors were observed.
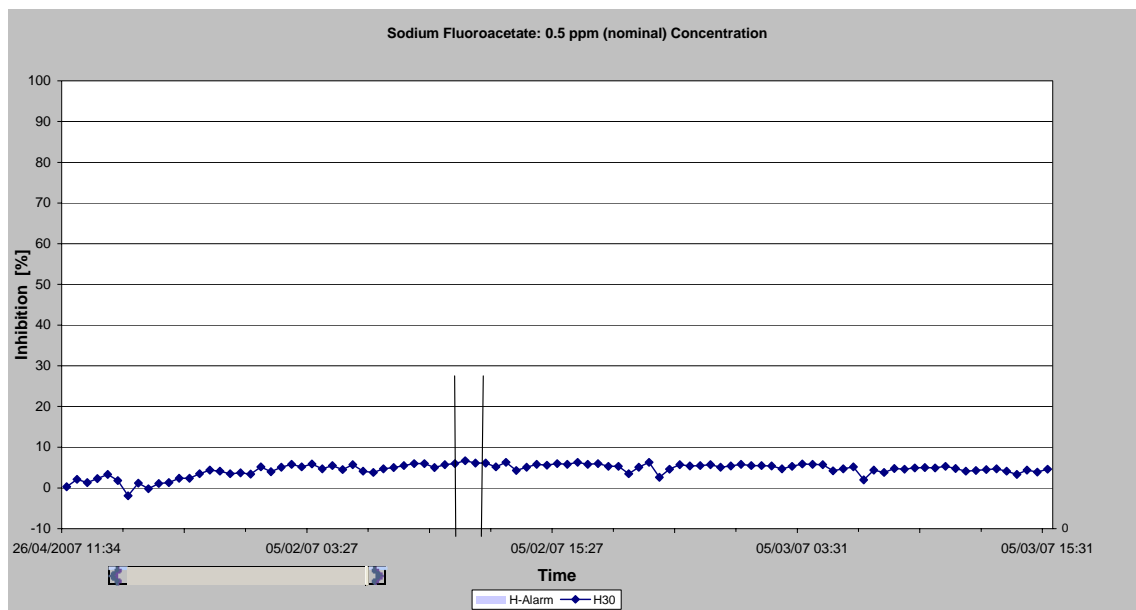
Figure 1: Responses of *Vibrio fischeri* to sodium fluoroacetate at 0.5 ppm (nominal) exposure concentration
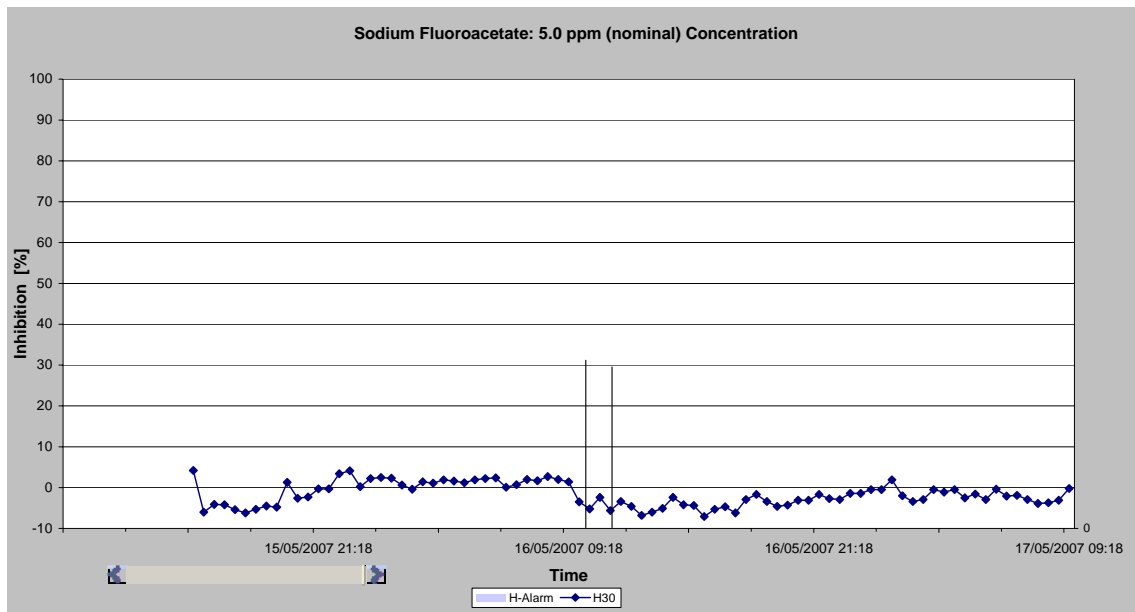

Figure 2: Responses of *Vibrio fischeri* to sodium fluoroacetate at 5.0 ppm (nominal) exposure concentration
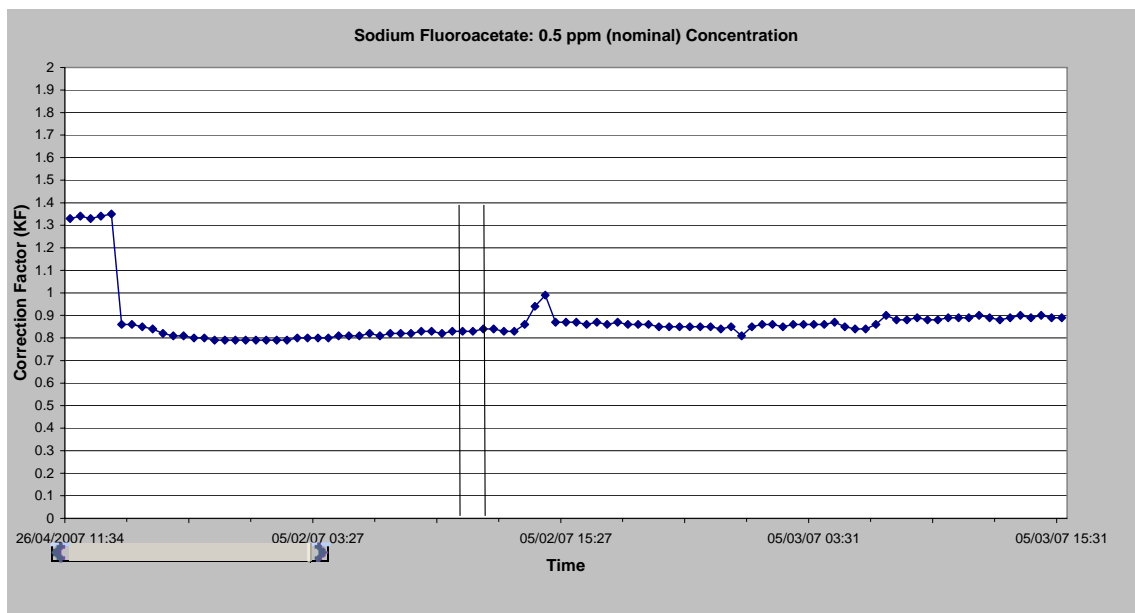

Figure 3: Variation of correction factors during 0.5 ppm (nominal) sodium fluoroacetate exposure concentration

Analytical confirmatory results using Ion Chromatograph show negligible concentrations of fluoroacetate in the samples collected during exposures. However, further bench-scale experiments proved that the fluoroacetate was in the sample but not detected due to interferences from high concentrations of sodium chloride in the sample.

Figures 4, 5 and 6 show the responses of *Vibrio fischeri* to 0.5, 0.05 and 0.005 (nominal) ppm cyanide exposure concentrations respectively. The 0.5 ppm cyanide created almost 100% inhibition. The response of *Vibrio fischeri* to 0.05 ppm cyanide was also very sensitive, the inhibitions created was around 50%. Although 0.005 ppm cyanide did not create any positive inhibitions, the responses were slightly higher during exposure in comparison with that during pre- and post-exposure periods. The high sensitivity of *Vibrio fischeri* to cyanide is explained as it disrupts the electron transport chain in the mitochondria membrane and prevents respiration (Christon & Rohrer, 2007). The correction factors were within the recommended range of 0.6 – 1.3 during the exposures of cyanide at the mentioned concentrations. Duplicate tests of the mentioned exposure concentrations showed similar responses and correction factors.
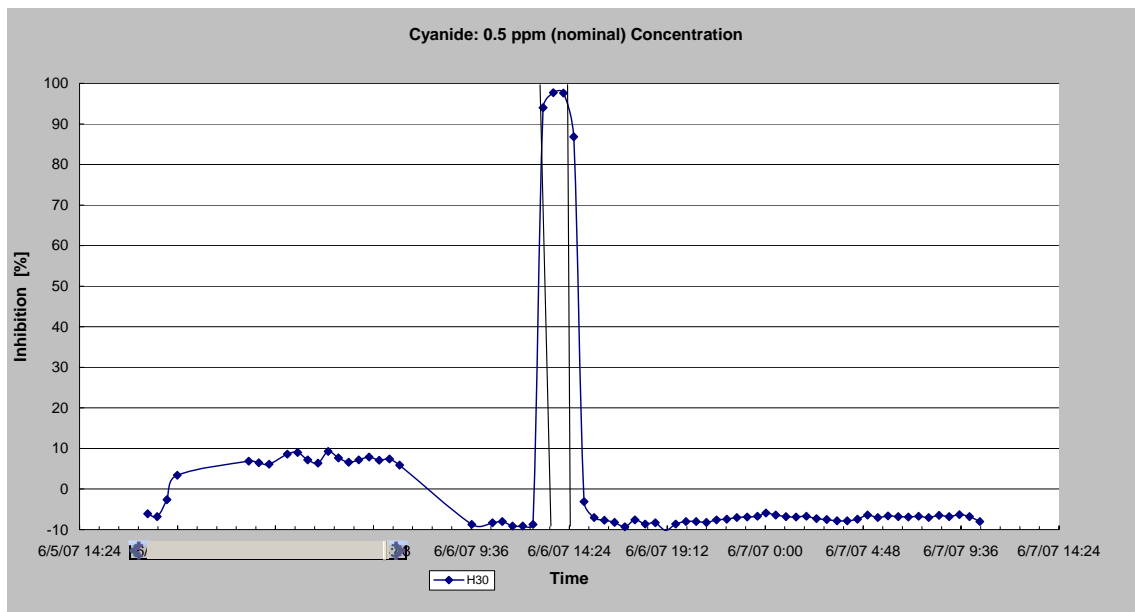

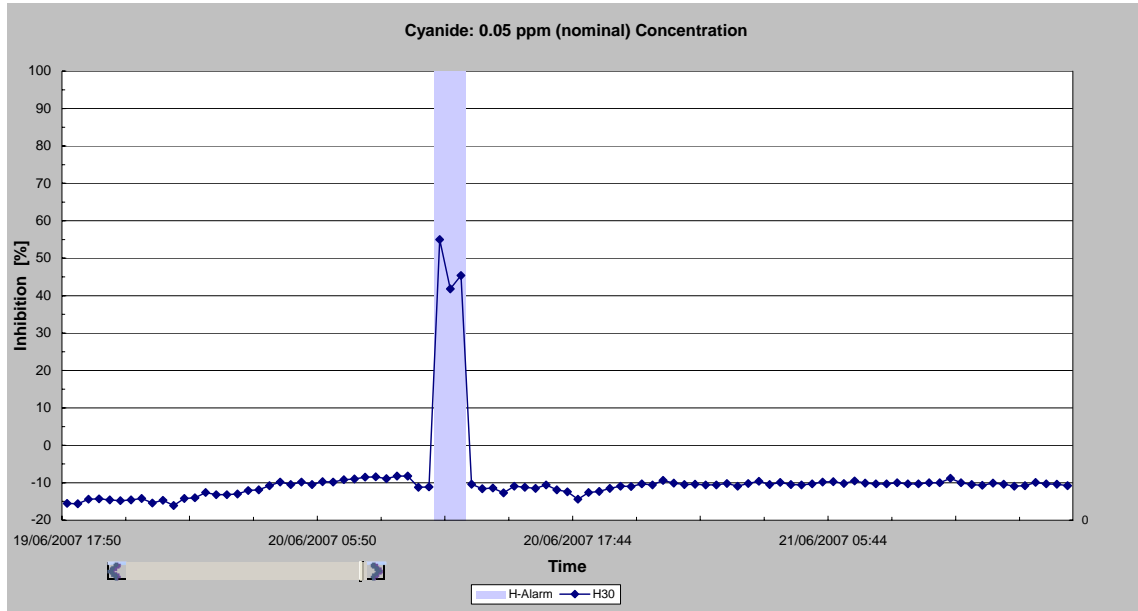**Figure 4: Responses of *Vibrio fischeri* to cyanide at 0.5 ppm (nominal) exposure concentration**

Figure 5: Responses of *Vibrio fischeri* to cyanide at 0.05 ppm (nominal) exposure concentration
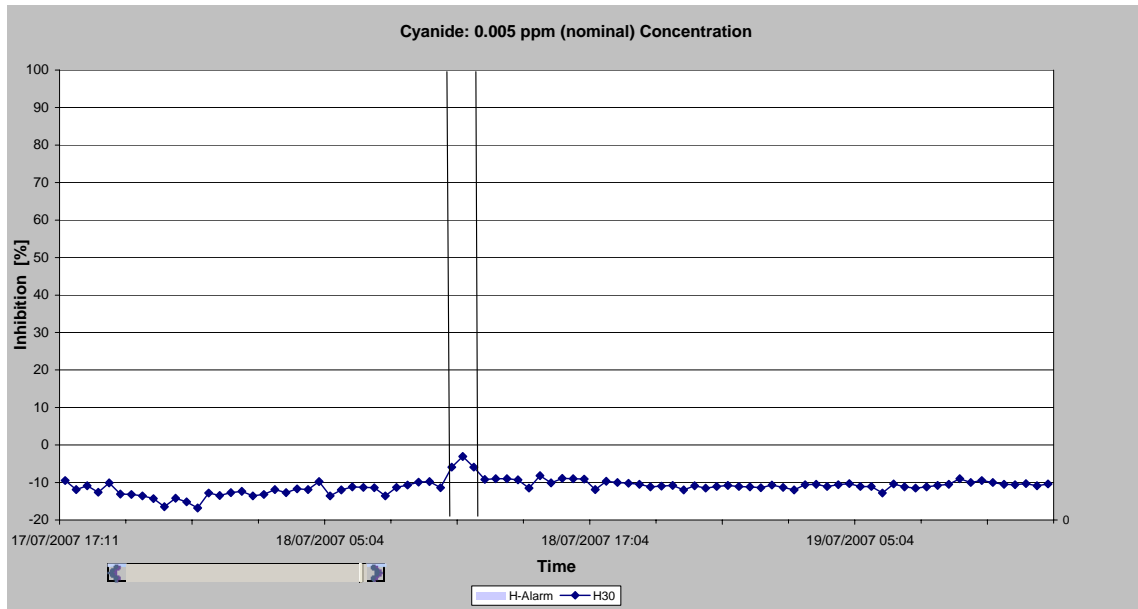

Figure 6: Responses of *Vibrio fischeri* to cyanide at 0.005 ppm (nominal) exposure concentration

Moldaenke ToxProtect System Response

The response recorded by the ToxcProtect during the 10.0 ppm (nominal) sodium fluoroacetate is shown in Figure 7. No significant increases in toxicity were observed during exposure in comparison with that during pre- and post-exposure periods. Also, no significant increases in toxicity were observed at 1.0 ppm (nominal) sodium fluoroacetate concentrations. Duplicate tests were conducted for the 1.0 ppm concentration also showed no significant increases in toxicity.

Analytical results by Ion Chromatograph showed the highest instrument test chamber concentration to be 9.539 ppm for the 10 ppm nominal sodium fluoroacetate. The highest instrument test chamber concentration for the 1.0 ppm nominal test was 1.208 ppm sodium fluoroacetate.
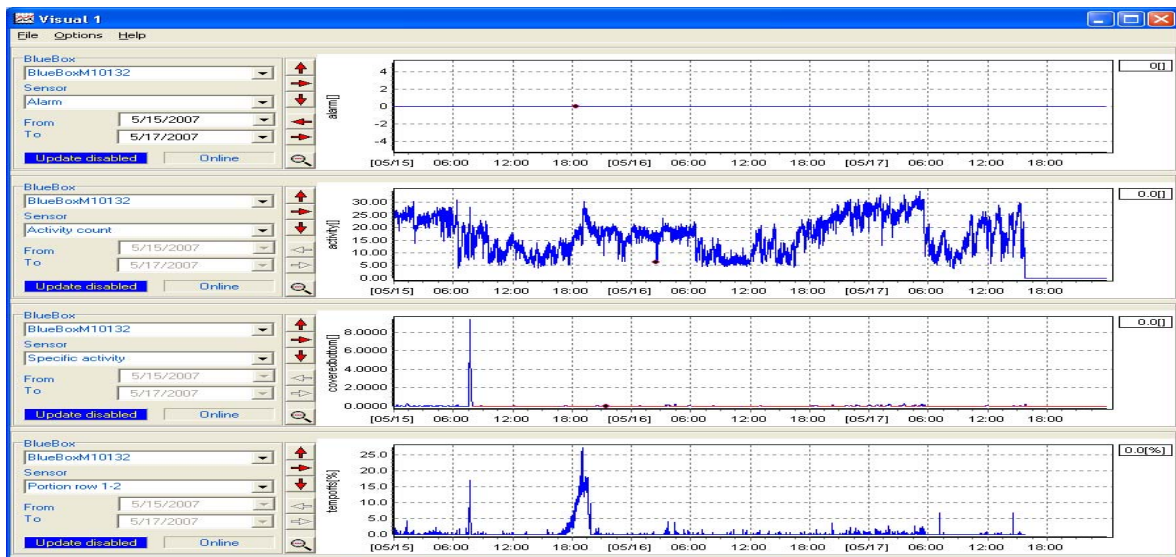

Figure 7: Responses of Fathead minnows to 10.0 ppm (nominal) sodium fluoroacetate exposure concentration
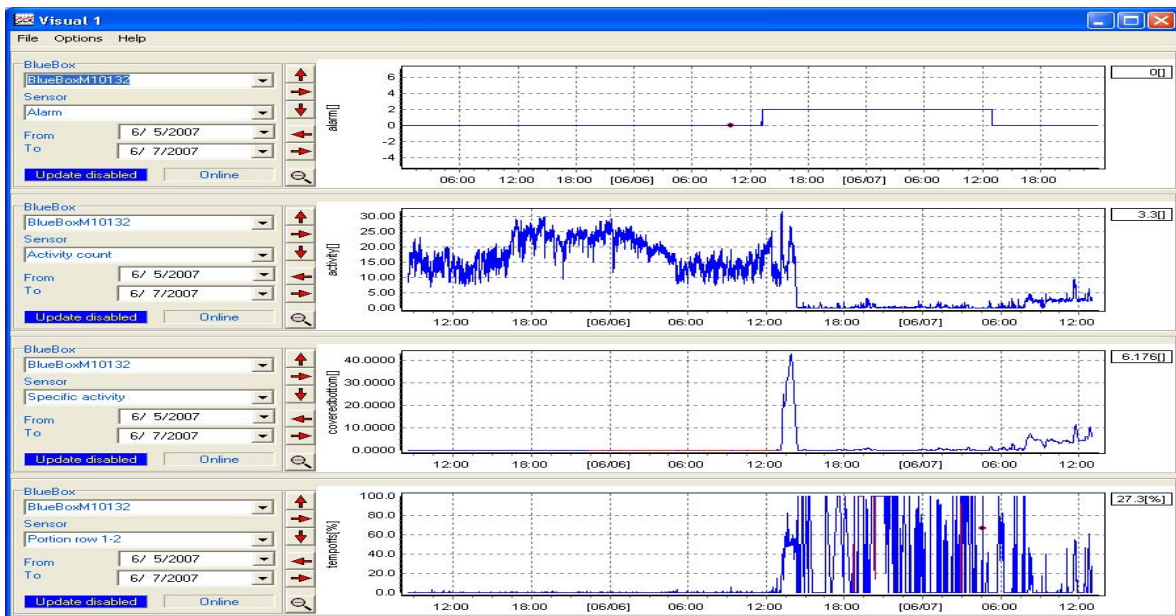
Figure 8: Responses of Fathead minnows to 1.0 ppm (nominal) cyanide exposure concentration

Figure 8 shows the response of the ToxProtect to 1.0 ppm (nominal) cyanide. The system alarmed within 21 minutes from start of injection due to high specific activity. The duplicate 1.0 ppm (nominal) cyanide test alarmed within 17 minutes from start of injection. With the 7 minute lag time for the contaminant to reach the system is taken into account, the instrument alarmed within 14 and 10 minutes respectively. Both tests had 100% mortality.

The system was also exposed to duplicate tests of 0.01 and 0.1 ppm (nominal) cyanide. No significant increases in toxicity level were observed during exposure as compared to pre- and post-baseline.

CONCLUSION
Based on the experimental data presented in this paper, the researchers conclude that both MicroLAN Toxcontrol and bbe-Moldaenke ToxProtect are capable of detecting low levels of cyanide injected into a distribution system. However, both OTMs were incapable of detecting considerably high levels of fluoroacetate in the DSS. The capability of OTMs to detect a particular toxicant in distribution systems depends on the physico-chemical activity of bioassay and nature of the toxicant. Interestingly, when the researchers reviewed the conventional online physcio-chemical instrument monitoring data (e.g., pH, ORP, Chlorine, turbidity and TOC), it appears that the sensitivity of those parameters was inverse. The physico-chemical sensors were more sensitive to sodium fluoroacetate but less sensitive to cyanide. OTM responses to cyanide injections were much more sensitive to the lower cyanide concentrations (0.1 and 0.01 ppm nominal). Further pilot-scale DSS studies using OTMs are underway to establish toxicant-specific OTM performance.

REFERENCES
Allan, I. J. 2006. A "toolbox" for biological and chemical monitoring requirements for the European Union Water Framework Directive. *Talanta*, 69:2:302.

Baldwin, I. G. & Kramer, K.J.M., 1994. *Biological Early Warning System (BWES).*, CRC Press, Boca Raton, Florida, U.S.A.

Baldwin, I. G. et al, 1994. Performance characteristics of a fish monitor for detection of toxic substances – II. Field trials. *Water Res*. 28:10:2201

Christison, T. T. & Rohrer. J. S. 2007. Direct determination of free cyanide in drinking water by ion chromatography with pulsed amperometric detection. *Jour. of Chromotography*, 1155:1:31

Gerhardt, A., 2005. Evidence for the stepwise stress model: *Gambusia holbrooki* and *Daphnia magma* under acid mine drainage and acidified reference water stress. *Environ. Sci. & Technol*. 39:11:4150

Hall, J. et al, 2007. On-line water quality parameters as indicators of distribution system contamination. *Jour. AWWA*, 99:1:66

International Life Science Institute (ILSI), 1999. *Early Warning Monitoring to Detect Hazardous Events in Water Supplies.* ISLI Press, Washington D.C., U.S.A.

Magnuson, M. L. et al, 2005. Responding to water contamination threats. *Env. Sci. & Technol*. 39:7:153A.

U.S. EPA, 2004. The water security research and technical support action plan. www.epa.gov/nhsrc/pubs/bookActionPlan031204.pdf.

 Zurita, J. L. et al, 2007. Ecotoxicological evaluation of sodium fluoroacetate on aquatic organisms and investigation on the effects of two fish cell lines, Chemosphere, 67:1:1.