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SCIENCE DIRECT
Biosensors and Bioelectronics 22 (2006) 18–27
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Selection of a battery of rapid toxicity sensors for drinking water evaluation

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Received 3 September 2005; received in revised form 7 November 2005; accepted 23 November 2005
Available online 10 January 2006

Abstract

Comprehensive identification of chemical contaminants in Army field water supplies can be a lengthy process, but rapid analytical methods suitable for field use are limited. A complementary approach is to directly measure toxicity instead of individual chemical constituents. Ten toxicity sensors utilizing enzymes, bacteria, or vertebrate cells were tested to determine the minimum number of sensors that could rapidly identify toxicity in water samples containing one of 12 industrial chemicals. The ideal sensor would respond at a concentration just exceeding the Military Exposure Guideline (MEG) level for the chemical (an estimated threshold for adverse effects) but below the human lethal concentration. Chemical solutions were provided to testing laboratories as blind samples. No sensors responded to deionized water blanks, and only one sensor responded to a hard water blank. No single toxicity sensor responded to more than six chemicals in the desired response range, and one chemical (nicotine) was not detected by any sensor with the desired sensitivity. A combination of three sensors (Microtox, the Electric Cell Substrate Impedance Sensing (ECIS) test, and the Hepatocyte low density lipoprotein (LDL) uptake test) responded appropriately to nine of twelve chemicals. Adding a fourth sensor (neuronal microelectrode array) to the test battery allowed detection of two additional chemicals (aldicarb and methamidophos), but the neuronal microelectrode array was overly sensitive to paraquat. Evaluating sensor performance using a standard set of chemicals and a desired sensitivity range provides a basis both for selecting among available toxicity sensors and for evaluating emerging sensor technologies. Recommendations for future toxicity sensor evaluations are discussed.

Keywords: Toxicity sensor; Test battery; Drinking water; Military Exposure Guideline; MEG

1. Introduction

Providing high quality drinking water free from chemical contaminants is important both for Army facilities in the United States as well as for Army personnel deployed around the world. During deployments, producing drinking water at a central source and transporting it to personnel in the field may improve quality control, but central water production requires valuable transportation assets. Decentralized water production makes it more difficult to ensure that water is free from chemical contamination, since options for rapid analysis of chemical contaminants are limited and more thorough analysis for a broad range of organic and inorganic chemicals can require complex instrumentation not readily available in many deployed situations. One alternative is to use biosensors that rapidly evaluate the toxicity of a whole water sample instead of measuring concentrations of specific chemical constituents. To this end, an effort was initiated to identify a battery of toxicity sensors that could increase the Army's capability to rapidly evaluate drinking water quality. The process described here provides an efficient method for screening available toxicity sensors and for selecting those best suited for inclusion in a toxicity testing system.

Previous efforts to evaluate groups of toxicity sensors for drinking water evaluation have focused on testing the sensors against single benchmark indicators of human health effects. The US Environmental Protection Agency Environmental Technology Verification (EPA ETV) Program tested eight commercially available rapid toxicity test systems against nine contaminants at concentrations at and below an estimated human lethal concentration (http://www.epa.gov/etv/verifications/vcenter1-27.html). In addition, several potential interfering chemicals

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doi:10.1016/j.bios.2005.11.019

associated with the water treatment process were tested at single concentrations likely to be encountered at water treatment facilities. Toxicity response thresholds were reported, with test responses above the human lethal concentration considered to be non-detects.

In a similar effort, toxicity sensors for drinking water protection were evaluated at the EILATox-Oregon Biomonitoring Workshop (Pancrazio et al., 2004). Eleven toxicity sensors were tested with up to 17 blind samples that included a wide range of toxic chemicals at maximum concentrations selected to be acutely toxic based on animal data. Sensors were scored as either detecting or being non-responsive to the blind samples, but because of testing limitations, 7 of the 11 toxicity sensors were tested at less than the maximum blind sample concentration, at dilutions ranging from 1:2 to 1:200. Because of time limitations at the workshop, not all the toxicity sensors were tested with all 17 blind samples.

Our effort drew from and expanded upon these sensor evaluations. We used blind samples and tested each sensor using a series of toxicant concentrations to define a common endpoint across all test chemicals. Both toxic chemicals and potential interferences were tested. Each chemical was tested in triplicate to provide an estimate of test variability. As with both the EPA ETV Program and the EILATox Workshop, we used an estimated human lethal dose as an upper limit for acceptability of toxicity sensor response, but we also added a lower response threshold to help assess false positive responses. This concentration range was established based on toxicity sensor performance requirements developed in coordination with Army users.

As a first step towards defining toxicity sensor performance requirements, an Army user group identified several specific Army scenarios that required water quality evaluations, ranging from water use by small units in the field to more established water treatment facilities found in rear areas and at garrisons. Equipment constraints increase substantially in field environments; size, weight, power consumption, and reagent requirements must decrease greatly. These logistical issues are being addressed as part of a formal Army toxicity sensor downselection process (ECBC DAT, 2004) and are not discussed further here. This paper describes toxicity sensor performance data required for the downselection process, including sensitivity to toxicants and test reproducibility.

An initial evaluation of the literature identified 38 potential toxicity sensor technologies that might contribute to one or more of the identified Army water use scenarios. An expert panel including individuals affiliated with Government, academia, or industry (including water utilities) selected the most promising sensors for further consideration. Some technologies were dropped from consideration for a variety of reasons, such as taking too long to produce a response (several hours or more) or being redundant with other technologies. Toxicity sensors with promise but at too early a stage of development to allow inclusion in a prototype system by the end of 2008 were included on a technology watch list. Ten of the 38 technologies were recommended for further testing to allow a comparison of their toxicity response characteristics.

The comparative evaluation of the 10 toxicity sensors included the following steps:

- Identification of toxicological benchmarks. Defining the concentration range that constitutes an acceptable sensitivity for the toxicity sensors.
- Selection of test chemicals. Identifying a set of common test chemicals that would permit meaningful comparisons among the toxicity sensors.
- Providing test chemical solutions as blind samples. Since toxicity sensors testing was conducted by several laboratories, common test solutions were sent out from a central source as blind samples.
- Defining performance metrics and analyzing test results. The goal was to identify the minimum number of toxicity sensors that would identify the maximum number of test chemicals with the desired level of sensitivity. Data on test reproducibility and failure rate were evaluated as well.

2. Methods

2.1. Toxicity sensors

The 10 toxicity sensors evaluated in this study by participating laboratories are described below.

2.1.1. Electric cell-substrate impedance sensing (ECIS)

The ECIS device measured toxicant-induced changes in the electrical impedance of a cell monolayer (Giaever and Keese, 1993; Keese et al., 1998). Bovine pulmonary artery endothelial cells from VEC Technologies (Rensselaer, NY) were seeded on eight small gold electrodes (Applied BioPhysics #8W1E) and grown to confluence. Current flowed between the smaller cell-covered electrode and a larger counter electrode through cell culture medium that bathed both electrodes. After background impedance was measured, the test or control sample was added and impedance was measured for up to 60 min; the actual response time was from 5–20 min for all but one of the chemicals tested.

2.1.2. Eclox

The Eclox acute toxicity sensor (Severn Trent Services, Colmar, PA) monitored a chemiluminescent oxidation–reduction reaction catalyzed by the plant enzyme horseradish peroxidase (Hayes and Smith, 1996; States et al., 2003). In contaminant-free water, light produced was detected by a photometer. In the presence of a contaminant, chemiluminescence was reduced. Reagents were added to a water sample in a disposable cuvette, and a photometer reading was taken after 4 min.

2.1.3. Hepatocyte low density lipoprotein (LDL) uptake

This sensor measured fluorescein isothiocyanate labeled LDL-uptake activity of human hepatoblastoma Hep G2 cells (Shoji et al., 1998, 2000). Cells were cultured in porous microcarriers at a high cell density and packed in a filter tip that had a hydrophobic membrane. Filter tips were then frozen at −85 °C