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

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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
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, vertebrate cells were testing 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.

Toxicity sensor; test battery; drinking water; Military Exposure Guideline; MEG
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.
and kept for at least 30 days until used. To analyze a water sample, the filter tips were thawed for 20 min and then held in culture media for 30 min. The culture media in the filter tip was then exchanged for a water sample in a concentrated culture medium containing fluorescently labeled LDL. The cells were exposed to the water sample for 2 h, then LDL that had not been taken into the cells was rinsed away, the cell membranes were lysed, and the fluorescently labeled LDL was released for fluorescent measurement. The fluorescence from LDL taken up during contaminant exposure was compared to fluorescence from cells exposed to contaminant-free water. (Since the performer of this assay was located in Japan, the test chemicals for this toxicity sensor were not provided as blind samples.)

2.1.4. Microtox

The Microtox acute toxicity sensor (Strategic Diagnostics, Inc., Newark, DE) measured changes in natural bioluminescence produced by the marine bacteria Vibrio fischeri (Bulich, 1979; States et al., 2003). Toxic substances decreased light output, which was measured using a photometer. Vibrio fischeri supplied in a standard freeze-dried (lyophilized) state were reconstituted in a salt solution. Luminescence readings were taken prior to adding the water samples and at 15 min after the addition.

2.1.5. Mitoscan

Mitoscan (Harvard Bioscience, Inc., Holliston, MA) measured enzyme activity of submitochondrial particles (SMPs), which are isolated vesicles of the inner membrane of bovine heart mitochondria containing membrane-bound enzymes associated with cellular electron transport and oxidative phosphorylation (Knobeloch et al., 1990). Two endpoints were monitored for each chemical tested: loss of nicotinamide adenine dinucleotide (NADH) using the electron transfer (ETR) protocol and production of NADH using the reverse electron transfer (RET) protocol. In both protocols, a concentrated reaction mixture and SMPs were added to the test samples. Then, either NADH (for ETR) or adenosine triphosphate (for RET) was added to the test samples and the loss or production of NADH was measured, respectively, over a 24-min exposure period.

2.1.6. Neuronal microelectrode array

This toxicity sensor evaluated changes in action potential (AP) activity, measured as mean spike rate, in a neuronal network via non-invasive extracellular recording (Pancrazio et al., 2003). Mouse frontal cortex neurons and glia grown on microelectrode arrays (MEAs) were purchased from the Center for Network Neuroscience at the University of North Texas (UNT). The cultures were isolated from embryonic day 14–15 mice and seeded onto arrays at UNT. After forming mature networks (3–4 weeks), the cultures on the MEAs were shipped to the Naval Research Laboratory (NRL) where the cultures were maintained in a standard laboratory incubator up to 5 days until use.

To initiate sample analysis, the neural network cartridges were equilibrated at 37 °C and pH 7.4. Then, a 15 min low-resolution recording was made of all 64 microelectrode channels in order to determine active electrode sites. Only networks with a minimum of eight active channels (defined as having a spike rate > 0.5 Hz) were used for these experiments. After determining the active channels present in the network, up to 16 were chosen for high resolution recording and contaminant testing. After the channels were selected, the network was attached to the biosensor’s fluidics system and fresh medium was perfused across the network. Experiments began after a stable baseline was observed for 30–60 min. For each chemical, the network was exposed to increasing test chemical concentrations at 30 min intervals.

2.1.7. Sinorhizobium meliloti toxicity test

This test used the bacterium S. meliloti, which readily reduced the tetrazolium dye MTT (Botsford, 2002). The normally light yellow dye turned dark blue when reduced by the bacteria; toxic chemicals inhibited reduction of the dye. The change in color was monitored with a spectrophotometer that measured the absorbance at 550 nm at zero time and after incubation for 20 min at 30 °C. The absorbance of the unknown samples was compared to distilled water controls.

2.1.8. SOS cytosensor system

This toxicity sensor measured changes in the optical appearance (pigment granule distribution) of fish chromatophores (Dierksen et al., 2004). Both direct effects of toxic agents (“direct optical responses”, DOR) and indirect effects (“indirect optical response”, IOR) were measured after a 60 min exposure period. IORs occurred when the normal responses of chromatophores to an added neurotransmitter (naphazoline) were disrupted. Chromatophores used were on scales from the Nile tilapia (Oreochromis niloticus).

Changes in chromatophores were recorded photographically. Test articles (comprised of eight chambers and eight fish scales) were mounted in a white-light illuminated framework above a 10× microscope lens. A digital camera coupled to the lens recorded the image of the fish scale in each chamber. Raw image data were converted to numerical metrics through digital color segmentation. Black melanophores, which to date are the most studied chromatophores, were demarcated for quantitative measurements. If preliminary inspection of the raw images showed that any of the other major classes of chromatophores (e.g., yellow xanthophores) varied significantly with experimental treatment, then that class also was demarcated and included as a class of interest in the following data processing steps. DOR and IOR values were measured and evaluated for each single fish scale by digital measurements. Toxicant concentrations that caused significant changes in chromatophore pigmentation as compared with control chromatophores were identified.

2.1.9. Toxi-Chromotest

The Toxi-Chromotest (Environmental Biodetection Products Inc., Brampton, Ont.) measured toxicant inhibition of the de novo synthesis of an inducible enzyme—β-galactosidase—in a highly permeable mutant of Escherichia coli (Reinhartz et al., 1987). The sensitivity of the test was enhanced by exposing the bacteria to stressing conditions and then lyophilizing them. Lyophilized bacteria were rehydrated in a cocktail containing a specific inducer of β-galactosidase and essential factors.
required for the recovery of the bacteria. The activity of the induced enzyme released by actively growing recovered cells was detected by the hydrolysis of a chromogenic substrate. Toxic materials interfered with the recovery process and thus with the synthesis of the enzyme and the resultant color reaction. After bacteria were exposed to a test chemical for 75 min, the chromogenic substrate was added. Samples were analyzed in a 96-well plate and the results were read with a plate reader.

2.1.10. ToxScreen II

The ToxScreen II test (Checklight Ltd., Israel) measured changes in light output from the naturally luminescent bacteria *Photobacterium leiognathi* (strain SB) (Ulitzur et al., 2002). The test included the use of two assay buffers; one favored the detection of heavy metals (Pro-Metal Buffer) while the other enhanced detection of organic contaminants (Pro-Organic Buffer).

To analyze a test sample, a suspension of lyophilized *P. leiognathi* was added to two aliquots of the test sample. One aliquot received Pro-Metal Buffer and the other received Pro-Organic Buffer. The luminescence of all the samples was measured after an exposure time of 1 h. In the absence of the toxic substances, the in vivo luminescence remained stable, while if there were toxic substances present, the luminescence decreased with respect to the controls.

2.2. Toxicological benchmark concentrations and test chemicals

To be useful, toxicity sensors should respond to chemical concentrations at levels relevant to human health. The Army user group recommended that, for a given chemical, a toxicity sensor should respond to concentrations exceeding the 7- to 14-day Military Exposure Guideline (MEG) levels, assuming water consumption of 15 L/day typical of arid environments (USACHPPM, 2004). Although MEGs are not enforceable military standards, the MEGs provide a reference point above which adverse effects may be expected. The estimated human lethal concentration (HLC) provided an upper limit for toxicity sensor response. HLCs were estimated from human lowest lethal dose (LDL0) calculated from either accidental human poisoning data or rodent LD50 data (TERA, 2004); when available, estimates based on human data were used. Doses were converted to concentrations assuming a 70 kg weight and 15 L of water consumed.

Test chemicals recommended by the Army user group (Table 1) represent different modes of toxic action and include some possible threat chemicals as well as chemicals found in source or product drinking waters that could cause inappropriate toxicity sensor responses (ammonia, copper, and residual chlorine). Deionized water and very hard water blanks were included as well. Test chemical stability in water was a criterion considered in the selection process. Although the importance of chemical mixtures in environmental contamination events is recognized, and the ability to directly measure the toxicity of mixtures is an important capability of toxicity sensors, the lack of MEGs and human lethality information for chemical mixtures led to a decision to focus this effort on single chemicals only.

<table>
<thead>
<tr>
<th>Chemical (concentration reported as)</th>
<th>Chemical abstract service (CAS) number</th>
<th>MEG (mg/L)</th>
<th>HLC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldicarb</td>
<td>116-06-03</td>
<td>0.005</td>
<td>0.047</td>
</tr>
<tr>
<td>Ammonia</td>
<td>7664-41-7</td>
<td>30</td>
<td>72.6</td>
</tr>
<tr>
<td>Copper sulfate (Cu²⁺)</td>
<td>7758-98-7</td>
<td>0.14</td>
<td>92.9</td>
</tr>
<tr>
<td>Mercuric chloride (Hg²⁺)</td>
<td>7487-94-7</td>
<td>0.001</td>
<td>24.1</td>
</tr>
<tr>
<td>Methamidophos</td>
<td>10265-92-6</td>
<td>0.002</td>
<td>1.4</td>
</tr>
<tr>
<td>Nicotine</td>
<td>54-11-5</td>
<td>0.13</td>
<td>1.87</td>
</tr>
<tr>
<td>Paraquat dichloride (cation)</td>
<td>4685-14-7</td>
<td>0.05</td>
<td>3</td>
</tr>
<tr>
<td>Phenol</td>
<td>108-95-2</td>
<td>3</td>
<td>65.3</td>
</tr>
<tr>
<td>Sodium arsenite (As⁺³)</td>
<td>7784-46-5</td>
<td>0.02</td>
<td>1.9</td>
</tr>
<tr>
<td>Sodium cyanide (CN⁻)</td>
<td>143-33-9</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Sodium hypochlorite (residual chlorine)</td>
<td>7681-52-9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sodium pentachlorophenate (anion)</td>
<td>131-52-2</td>
<td>0.14</td>
<td>65.3</td>
</tr>
<tr>
<td>Toluene</td>
<td>108-88-3</td>
<td>1.00</td>
<td>2800</td>
</tr>
<tr>
<td>Deionized water blank</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Very hard water blank (Marking and Dawson, 1973)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

2.3. Blind sample preparation and testing

Test solutions were prepared at and shipped as coded samples to participating laboratories from Battelle Memorial Institute (Columbus, OH). Chemicals were obtained from Fisher (Fairlawn, NJ) or ChemService (West Chester, PA). Residual chlorine samples were prepared in chlorine demand-free water. As a quality control measure, the actual concentrations of each solution prepared and shipped to the participating laboratories were determined by analytical measurement at the US Army Center for Environmental Health Research (Ft. Detrick, MD). The endpoint concentrations reported were corrected for the difference between the nominal and actual concentration of the stock solution supplied each laboratory. In most cases, the difference was less then 15%.

The coded solutions were shipped in random order to the participating laboratories. With the exception of toluene, the stability of the samples over a 2-week period had been confirmed.
prior to the start of this study. Because of its volatility, toluene was to be analyzed as soon as possible after receipt. While the stability of the nicotine samples was confirmed, previous experience with nicotine caused concern about its degradation in water. Because of this, the nicotine samples were required to be analyzed as soon as possible after receipt. Otherwise, the sample maximum holding time prior to analysis was two weeks. Participating test laboratories were not told of the identity of the chemicals or of the MEG concentration levels, but they were told the concentration of the samples they received so accurate endpoint concentrations could be reported. For each chemical that was detected during initial range-finding tests, participating laboratories performed three definitive tests. If no response was observed for the undiluted blind sample, the original sample concentration was repeated in triplicate to confirm the lack of response.

2.4. Data analysis

The response endpoint (RE) for the toxicity sensors was the EC50 value generated from a linear regression of the results, except for the ECIS and SOS cytosensor systems for which the RE was a minimum detectable concentration determined from statistical hypothesis testing. A simple way of scoring toxicity sensor performance was to add up the number of chemicals each sensor detected between the MEG level and the HLC. In addition, a scoring system was used to determine the proximity of the RE to the MEG and HLC. The score for an RE above the MEG, reaching 0.0 at the HLC. The score for an RE above the HLC or below the MEG was 0.0. An ideal sensor (one for which each RE was at the MEG level) would have a total score of 12.

Further analyses of toxicity sensor performance considered whether the sensor responded to the deionized or hard water blanks or to residual chlorine. Test reproducibility was determined by calculating the median coefficient of variation for those blanks or to residual chlorine. Test reproducibility was determined by calculating the median coefficient of variation for those chemicals or of the MEG concentration levels, but they were told the concentration of the samples they received so accurate endpoint concentrations could be reported. For each chemical that was detected during initial range-finding tests, participating laboratories performed three definitive tests. If no response was observed for the undiluted blind sample, the original sample concentration was repeated in triplicate to confirm the lack of response.

3. Results and discussion

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Microtox</th>
<th>ECIS</th>
<th>Hepatocyte LDL uptake</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldicarb</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ammonia</td>
<td>–</td>
<td>1.00</td>
<td>–</td>
<td>1.00</td>
</tr>
<tr>
<td>Arsenic</td>
<td>–</td>
<td>0.20</td>
<td>0.16</td>
<td>0.20</td>
</tr>
<tr>
<td>Copper</td>
<td>0.81</td>
<td>0.40</td>
<td>0.13</td>
<td>0.81</td>
</tr>
<tr>
<td>Cyanide</td>
<td>1.00</td>
<td>–</td>
<td>–</td>
<td>1.00</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.49</td>
<td>0.14</td>
<td>0.01</td>
<td>0.49</td>
</tr>
<tr>
<td>Methamidophos</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nicotine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Paraquat</td>
<td>–</td>
<td>–</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.28</td>
<td>–</td>
<td>0.49</td>
<td>0.49</td>
</tr>
<tr>
<td>Pentachlorophenate</td>
<td>0.58</td>
<td>0.39</td>
<td>0.29</td>
<td>0.58</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.69</td>
<td>0.46</td>
<td>–</td>
<td>0.69</td>
</tr>
<tr>
<td><strong>Combined score</strong></td>
<td><strong>3.85</strong></td>
<td><strong>2.58</strong></td>
<td><strong>1.35</strong></td>
<td><strong>5.54</strong></td>
</tr>
<tr>
<td><strong>Chemicals detected</strong></td>
<td><strong>6</strong></td>
<td><strong>6</strong></td>
<td><strong>6</strong></td>
<td><strong>9</strong></td>
</tr>
</tbody>
</table>
Table 4

Response endpoints (REs) for each sensor and chemical tested

<table>
<thead>
<tr>
<th>Sensor/Metric</th>
<th>Aldicarb</th>
<th>NH₃</th>
<th>As³⁺</th>
<th>Ca²⁺</th>
<th>CN⁻</th>
<th>Hg²⁺</th>
<th>Methamidophos</th>
<th>Nicotine</th>
<th>Paraquat</th>
<th>Phenol</th>
<th>Pentachlorophenate</th>
<th>Toluene</th>
<th>Chlorine</th>
<th>DI water</th>
<th>Very hard water</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECIS</td>
<td>45.8</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>61.0</td>
<td>347</td>
<td>27.7</td>
<td>1.86</td>
<td>11.5</td>
<td>0.28</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Hepatocyte LDL uptake</td>
<td>241</td>
<td>30.0(1.0)</td>
<td>0.77(0.20)</td>
<td>6.93(0.40)</td>
<td>NR</td>
<td>6.00(0.14)</td>
<td>89.2</td>
<td>640</td>
<td>NR</td>
<td>73.3</td>
<td>6.12(0.39)</td>
<td>72.5</td>
<td>0.46</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>SOS cytoSensor</td>
<td>NR</td>
<td>NR</td>
<td>0.90(0.16)</td>
<td>40.2(0.13)</td>
<td>6.91</td>
<td>24.0(0.01)</td>
<td>4.66</td>
<td>15.4</td>
<td>0.95(0.28)</td>
<td>14.6</td>
<td>0.49</td>
<td>11.3</td>
<td>0.29</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Neuronal microelectrode array</td>
<td>0.0074</td>
<td>10.2</td>
<td>5.6</td>
<td>ND</td>
<td>5.20</td>
<td>1.08(0.18)</td>
<td>700</td>
<td>0.00002</td>
<td>5.94(0.78)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mitoscan ETR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0.29(0.89)</td>
<td>0.052</td>
<td>0.14(0.51)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>662</td>
<td>19.6(0.20)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>ToxScreen II Metals</td>
<td>NR</td>
<td>NR</td>
<td>5.58</td>
<td>1.19(0.67)</td>
<td>&lt;BS</td>
<td>0.09(0.55)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>54.6</td>
<td>877</td>
<td>18.8</td>
<td>0.20</td>
<td>NR</td>
<td>1.65</td>
</tr>
<tr>
<td>ToxScreen II Organics</td>
<td>NR</td>
<td>NR</td>
<td>8.63</td>
<td>200</td>
<td>0.12</td>
<td>0.04(0.63)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>93.8</td>
<td>27.4(0.28)</td>
<td>0.008</td>
<td>159(0.36)</td>
<td>0.20</td>
<td>NR</td>
</tr>
<tr>
<td>Toxi-Chromotest</td>
<td>NR</td>
<td>NR</td>
<td>3.80</td>
<td>NR</td>
<td>0.33</td>
<td>0.12(0.53)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>152</td>
<td>1000</td>
<td>2.0(0.57)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>S. melli i toxicity test</td>
<td>NR</td>
<td>132</td>
<td>179</td>
<td>3.08</td>
<td>0.08(0.56)</td>
<td>105</td>
<td>926</td>
<td>8.50</td>
<td>1.66</td>
<td>1.03(0.67)</td>
<td>268(0.30)</td>
<td>NR</td>
<td>NR</td>
<td>1.67</td>
<td></td>
</tr>
<tr>
<td>Mitoscan RET</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0.02</td>
<td>NR</td>
<td>0.09(0.55)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>303</td>
<td>0.06</td>
<td>NR</td>
<td>1.66</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Eclos</td>
<td>NR</td>
<td>NR</td>
<td>260</td>
<td>88.9(0.01)</td>
<td>7.15</td>
<td>28.6</td>
<td>NR</td>
<td>167</td>
<td>744</td>
<td>0.79</td>
<td>NR</td>
<td>0.38</td>
<td>0.38</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Blind sample concentration</td>
<td>500</td>
<td>300</td>
<td>200</td>
<td>1400</td>
<td>20</td>
<td>100</td>
<td>1300</td>
<td>500</td>
<td>300</td>
<td>140</td>
<td>500</td>
<td>10</td>
<td>NA</td>
<td>300</td>
<td></td>
</tr>
</tbody>
</table>

Concentrations are in mg/L; responses between the MEG concentration and the HLC are shaded and corresponding calculated scores are shown in parentheses.

a NR—no response at the blind sample concentration (the maximum concentration that could be tested).
b Testing was not done using blind samples.
c G. Gross, University of North Texas (unpublished data).
d ND—not determined; test data not available.
e NA—not applicable.
f Hardness, reported as mg/L as CaCO₃.
were detected when the results were combined, so that 9 of the
12 chemicals tested were detected in the MEG–HLC range
by the three sensors taken together. The three chemicals not
detected were aldicarb, nicotine, and methamidophos. All three
are neurotoxicants, and both aldicarb and methamidophos are
acetylcholinesterase inhibitors.

A more comprehensive summary of toxicity sensor responses
is shown in Table 4. The sensors are listed in the same order as
in Table 2, and sensor responses within the MEG–HLC range
are shaded. Because the Microtox, ECIS, and the Hepatocyte
LDL uptake systems together identified 9 of 12 chemicals in the
desired sensitivity range, the question is whether any of the other
toxicity sensors, if added to this potential toxicity test battery,
would either detect additional chemicals or respond closer to the
MEG concentrations.

Although none of the tested sensors detected nicotine, the
neuronal microelectrode array was able to detect aldicarb and
methamidophos with the desired sensitivity. Higher individual
sensor scores than for Microtox, ECIS, or the Hepatocyte LDL
uptake systems were found for four other chemicals: copper
(Mitoscan ETR – 0.89 versus Microtox – 0.81); mercury (sev-
eral sensors with the highest being ToxScreen II Organics – 0.63
versus Microtox – 0.49); phenol (neuronal microelectrode array
– 0.78 versus Hepatocyte LDL uptake – 0.49); pentachlorophenate
(S. meliloti Test – 0.67 versus Microtox – 0.58). These score differences
corresponded to concentrations of approximately 0.2 mg/L for copper
(Mitoscan ETR – 0.89 versus Microtox – 0.81); mercury (sev-
eral sensors with the highest being ToxScreen II Organics – 0.63
versus Microtox – 0.49); phenol (neuronal microelectrode array
– 0.78 versus Hepatocyte LDL uptake – 0.49); pentachlorophenate.
These relatively small sensitivity differences do not in and of themselves support
adding any sensors to the three sensors previously evaluated
together.

Table 5 summarizes how increasing the number of toxicity
sensors adds to the number of chemicals detected within the
MEG–HLC range. Sensors are listed starting with the highest
scoring Microtox (Table 2), followed by sensors that provide
the maximum increase in the number of additional chemicals
detected when combined with the preceding sensors. None of
the other sensors besides these four provided additional detection
capabilities. Although the neuronal microelectrode array
deserves further consideration because of its ability to detect
aldicarb and methamidophos in the desired sensitivity range,
the neurons also responded to paraquat at a concentration over
three orders of magnitude below the MEG.

The issue of sensitivity to toxicants must be considered within
the perspective of the benchmark concentrations. In particular,
the HLCs are only estimates, based on frequently incomplete
or absent human poisoning data or extrapolations from rodent
LD50 data. For aldicarb, nicotine, and cyanide, the MEG–HLC
range is less than a factor of 10, which may reflect conserva-
tive assumptions made in developing the HLCs. In addition,
human lethal doses were converted to concentrations using a
15 L volume of water, to be consistent with the daily water con-
sumption appropriate to the MEG values chosen by the Army
user group. However, a more accurate estimation of a lethal
concentration would have included a toxicokinetic analysis to
account for variations in absorption, metabolism, and excretion
for a lethal chemical dose consumed over time in 15 L of water.

Table 6 compares the response concentrations identified in
this study to available literature values. Caution should be used
when making comparisons with literature data, given the diffi-
culty in ensuring comparable methodologies and biological test
systems. For a given toxicity sensor and test chemical, most of
the literature values and the data from this study are within the
same response category. That is, the responses are either both
within the MEG–HLC range or both outside the range. How-
ever, a few literature values differ substantially from the toxicity
sensor data compiled for this study, and many of the greatest
differences are found with the ToxScreen II or S. meliloti tox-
icity tests. One contributing factor might be the relatively high
variability in these test systems, as discussed below.

Data on other factors besides sensitivity to toxicants that
could help discriminate among toxicity sensors were collected
as part of this study (Table 7). Residual chlorine concentration
in military water supplies are typically maintained at 2 mg/L,
and five of the sensors responded to residual chlorine levels in
this range, including Microtox and the Hepatocyte LDL uptake
sensors. Although it would be convenient if the sensors would
not respond to residual chlorine, chlorine can be removed prior
to analysis using sodium thiosulfate or another similar reduc-
ing agent (Yonkos et al., 2001). There was only one instance of
response to either the DI water blank or the hard water sam-
ple. The S. meliloti toxicity test produced a false indication
of toxicity when exposed to very hard water, but S. meliloti is
known to be sensitive to calcium and other divalent cations in
water (Botsford, 2002). Although adding a chelating agent could
remove divalent cation responses, this would likely reduce sen-
titivity to toxic metals of concern, such as Hg2+. The median
coefficients of variation (CVs) provide a rough estimate of the
reproducibility of the REs generated by the toxicity sensors. CV
values ranged from 6 to 54% with all but three sensors being less
than 20%. The ToxScreen and S. meliloti toxicity tests had the
highest CVs. The CVs of the toxicity sensors in the candidate
test battery were less than 25%.

Most of the sensors had a very low reported failure rate, but
the neuronal microelectrode array system had approximately a
65% failure rate, almost entirely due to shipping problems. When

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Table 5
Value of additional toxicity sensors for increasing the number of chemicals detected

<table>
<thead>
<tr>
<th>Added sensor</th>
<th>Cumulative chemicals detected</th>
<th>Additional chemicals detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtox</td>
<td>6</td>
<td>Copper, cyanide, mercury, phenol, pentachlorophenate, toluene</td>
</tr>
<tr>
<td>Hepatocyte LDL uptake</td>
<td>8</td>
<td>Arsenic, paraquat</td>
</tr>
<tr>
<td>Neuronal microelectrode array</td>
<td>10</td>
<td>Aldicarb, methamidophos</td>
</tr>
<tr>
<td>ECIS</td>
<td>11</td>
<td>Ammonia</td>
</tr>
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</table>
Table 6
Comparison of toxicity sensor responses with literature data

<table>
<thead>
<tr>
<th>Toxicity testa</th>
<th>Data source</th>
<th>As^3+</th>
<th>Cu^2+</th>
<th>CN^-</th>
<th>Hg^2+</th>
<th>Nicotine</th>
<th>Paraquat</th>
<th>Phenol</th>
<th>Pentachlorophenate</th>
<th>Toluene</th>
<th>Chlorine</th>
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</thead>
<tbody>
<tr>
<td>Microtox</td>
<td>This study</td>
<td>24.7</td>
<td>0.47</td>
<td>2.00</td>
<td>0.18</td>
<td>61.0</td>
<td>347</td>
<td>27.7</td>
<td>1.86</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td>Literature</td>
<td>2784b</td>
<td>0.64b</td>
<td>1.77bc</td>
<td>0.03bc</td>
<td>36.3bc</td>
<td>436b</td>
<td>342d</td>
<td>0.70d</td>
<td>19.7d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuronal microelectrode array</td>
<td>This study</td>
<td>5.6</td>
<td>0.5–10e</td>
<td></td>
<td>1–10e</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitoscan ETR</td>
<td>This study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>662</td>
<td>133f</td>
<td></td>
</tr>
<tr>
<td>Literature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToxScreen II Metals</td>
<td>This study</td>
<td>1.19</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Literature</td>
<td>0.007f</td>
<td>0.03f</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToxScreen II Organics</td>
<td>This study</td>
<td>8.63</td>
<td>200</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Literature</td>
<td>18g</td>
<td>0.02g</td>
<td>0.003f</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tox-Chromotest</td>
<td>This study</td>
<td>3.80</td>
<td>0.33</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Literature</td>
<td>0.88</td>
<td>0.17b</td>
<td>0.12h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. meliloti toxicity test</td>
<td>This study</td>
<td>89.2</td>
<td>179</td>
<td>3.08</td>
<td>0.08</td>
<td>926</td>
<td>8.50</td>
<td>1.66</td>
<td>1.03</td>
<td>268</td>
<td></td>
</tr>
<tr>
<td>Literature</td>
<td>1.7b</td>
<td>0.44i</td>
<td>5.75j</td>
<td>0.01i</td>
<td>990i</td>
<td>47.9j</td>
<td>1214j</td>
<td>0.14j</td>
<td>217j</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitoscan RET</td>
<td>This study</td>
<td>0.02</td>
<td></td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
<td>303</td>
<td>0.06</td>
<td></td>
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</tr>
<tr>
<td>Literature</td>
<td>0.38</td>
<td>0.13f</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>133f</td>
<td>0.08f</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EcloX</td>
<td>This study</td>
<td>88.9</td>
<td>7.15</td>
<td>28.6</td>
<td></td>
<td></td>
<td></td>
<td>0.79</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Literature</td>
<td>242i</td>
<td>0.01j</td>
<td>16j</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.0j</td>
<td>0.03j</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparisons are made for test results reported in Table 4 for which endpoint values could be computed and literature values were available. Shaded values are those within MEG–HLC range for the chemical.

a No comparable literature data were found for any chemical tested with the ECIS, Hepatocyte LDL uptake, or SOS cytosensor tests.
b Clemedson et al. (1996).
c Five-minute EC50 data; 15-min data not available.
e O’Shaughnessy et al. (2004).
f Blondin et al. (1989).
g Ulitzur et al. (2002).
h Reinhartz et al. (1987).
i Botsford (2002).
j Sawcer and Thorpe (2001); data are reported for an assay said to produce results equivalent to EcloX.

Table 7
Other factors differentiating toxicity sensors

<table>
<thead>
<tr>
<th>Toxicity sensor</th>
<th>RE for chlorine (mg/L)</th>
<th>False positive for hard water</th>
<th>False positive for DI water</th>
<th>Overall median CV</th>
<th>Failure rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtox</td>
<td>0.28</td>
<td>No</td>
<td>No</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>ECIS</td>
<td>&gt;10</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>17</td>
</tr>
<tr>
<td>Hepatocyte LDL uptake</td>
<td>5.46</td>
<td>No data</td>
<td>No data</td>
<td>12.7</td>
<td>5–10</td>
</tr>
<tr>
<td>SOS cytosensor system</td>
<td>&gt;10</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>2</td>
</tr>
<tr>
<td>Neuronal microelectrode array</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>11</td>
<td>65</td>
</tr>
<tr>
<td>Mitoscan (ETR)</td>
<td>&gt;10</td>
<td>No</td>
<td>No</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>S. meliloti toxicity test</td>
<td>&gt;10</td>
<td>Yes</td>
<td>No</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>ToxScreen II-Metals</td>
<td>1.65</td>
<td>No</td>
<td>No</td>
<td>54</td>
<td>10</td>
</tr>
<tr>
<td>ToxScreen II-Organics</td>
<td>0.20</td>
<td>No</td>
<td>No</td>
<td>31</td>
<td>10</td>
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<tr>
<td>Toxi-Chromotest</td>
<td>&gt;10</td>
<td>No</td>
<td>No</td>
<td>14</td>
<td>0</td>
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<tr>
<td>Mitoscan (RET)</td>
<td>1.66</td>
<td>No</td>
<td>No</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>EcloX</td>
<td>0.38</td>
<td>No</td>
<td>No</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

the problems were corrected, the failure rate was only 6%. The ECIS had a failure rate of 17%, which was due primarily to an improperly functioning incubator; the failure rate excluding incubator problems was 6.7%.

4. Conclusions

The analysis of the twelve chemicals using 10 different sensors resulted in the recommended combination of Microtox,
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5. Recommendations

The following steps are recommended for future efforts to identify toxicity sensors suitable for use in drinking water evaluations:

- Clearly define the intended application for the toxicity sensors, with input from the user community if possible.
- Based on the intended use(s), define acceptable sensitivity ranges for sensor response. Both an upper and a lower concentration boundary should be consistently defined.
- Evaluate the toxicity sensors with chemicals representing modes of toxic action of concern. If several laboratories are involved in sensor testing, provide test solutions as blind samples and take steps to ensure sample stability throughout the testing period.
- Apply consistent metrics for determining the relative usefulness of the toxicity sensors for the intended purpose. The simple metrics used in this study should be adequate for a screening evaluation. For more definitive performance evaluations, other approaches such as Receiver Operator Characteristic (ROC) curves (Beck and Shultz, 1986) may be useful.

Acknowledgements

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