EDGEWOOD

CHEMICAL BIOLOGICAL CENTER

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

ECBC-TR-477

INITIAL TECHNOLOGY ASSESSMENT FOR THE ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM

Scott Kooistra
John Walther

ADVANCED PLANNING AND INITIATIVES DIRECTORATE

August 2006

Approved for public release;
distribution is unlimited.

20060920005

ABERDEEN PROVING GROUND, MD 21010-5424

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE *(DD-MM-YYYY)* XX-08-2006	**2. REPORT TYPE** Final	**3. DATES COVERED** *(From - To)* Jan 2004 - Jun 2004
4. TITLE AND SUBTITLE Initial Technology Assessment for the Environmental Sentinel Biomonitor (ESB) System		**5a. CONTRACT NUMBER** **5b. GRANT NUMBER** **5c. PROGRAM ELEMENT NUMBER**
6. AUTHOR(S) Kooistra, Scott; and Walter, John		**5d. PROJECT NUMBER** None **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES) DIR, ECBC, ATTN: AMSRD-ECB-AP-I, APG, MD 21010-5424		**8. PERFORMING ORGANIZATION REPORT NUMBER** ECBC-TR-477
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Center for Environmental Health Research, 568 Doughten Drive, Fort Detrick, MD 21702-5010		**10. SPONSOR/MONITOR'S ACRONYM(S)** **11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The U.S. Army Center for Environmental Health Research (USACEHR), with support from the U.S. Army client organizations and funding from U.S. Army Science and Technology Objective (STO) IV.ME.2004.03, is developing an Environmental Sentinel Biomonitor (ESB) system to provide rapid toxicity identification for a broad spectrum of chemicals in water. A critical initial phase of the STO is to test and evaluate toxicity sensor technologies (also called ESB system technologies). Because there are a number of potentially feasible technologies that could meet the goals of the ESB program, a downselect will be performed to evaluate these technologies and select the most promising technologies for further development as part of an ESB system. The methodology and process to complete the downselect was developed in FY04 with user representatives and technology experts. Because critical data was not initially available, the final ESB system technology downselection was postponed until the data became available. Tests to generate the needed data will be complete in FY05. The final ESB system technology downselection, using the methodology and process developed in FY04, will occur after the test data becomes available. The methods and processes used in the technology downselection produce repeatable, defensible, and justifiable investment decisions.

15. SUBJECT TERMS

Detection rapidity of response and sensitivity	Human lethal dose	Decision analysis
Short term military exposure guidelines	Threshold requirements	Concepts of use
Multi-attribute utility model	Water monitoring	Downselect
Toxic industrial chemicals		

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Sandra J. Johnson
U	U	U	U	72	**19b. TELEPHONE NUMBER** *(include area code)* (410) 436-2914

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Blank

PREFACE

The work described in this report was authorized under the U.S. Army Science and Technology Objective (STO) IV.ME.2004.03. The work described in this report was started in January 2004 and completed in June 2004.

This report was prepared in response to a request from the U.S. Army Center for Environmental Health Research (USACEHR) to develop a methodology to evaluate Environmental Sentinel Biomonitor (ESB) technologies and then to evaluate and downselect the most appropriate ESB technologies to further develop into an ESB system.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

Registered users should request additional copies of this report from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

This report has been approved for public release.

Blank

CONTENTS

1. OVERVIEW 1

1.1 Introduction 1
1.2 Background 1
1.2.1 Problem Being Addressed/Problem Definition 1
1.2.2 How the ESB System Addresses the Problem 1
1.2.3 ESB Program Objectives and Goals 1
1.2.4 Desire to Leverage with Other Technologies 2

2. FY04 ESB SYSTEM INITIAL TECHNOLOGY ASSESSMENT RESULTS 2

2.1 Study/Evaluation Team Formed 2
2.2 ESB System Use Concepts/Applications 3
2.2.1 Six Scenarios Considered 4
2.2.1.1 Individual/Small Team Scenario 4
2.2.1.2 Future Combat System - Manned Ground Vehicle Scenario 4
2.2.1.3 Field/Contingency Scenarios 4
2.2.1.3.1 Grab Sample to Test Raw or Treated Water Scenario 4
2.2.1.3.2 Continuous Flow Sample to Test Treated Water Scenario 5
2.2.1.4 Fixed-Base Scenarios 5
2.2.1.4.1 Grab Sample to Test Raw or Treated Water Scenario 5
2.2.1.4.2 Continuous Flow Sample to Test Raw or Treated Water Scenario 5
2.3 Technical Requirements Defined 5
2.4 Candidate ESB System Technologies Identified and Partially Described 6
2.5 Expert Team Determines Additional Testing Is Needed 6
2.6 Interim Downselection Process Completed 6
2.6.1 Overview Description of Downselection Evaluation Model Compontents and How Model is Being Developed 6
2.6.2 Development To Date of Downselection Evaluation Model 7
2.6.3 Expert Panel Evaluated Technologies Using a High-Level Qualitative Model 7
2.7 Selected Training/Test Chemical Set, Developed Test Plan, and Started Testing Nine Technologies 9

3. FY05 PLANS TO COMPLETE THE ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNOLOGY DOWNSELECTION PROCESS 9

3.1 Complete Data Collection for Nine "Finalist" Technologies 9
3.2 Complete Evaluation Model 9
3.3 Evaluate Nine Technologies 9
3.4 Complete Analysis and Produce Final Report 10

APPENDIXES

A. ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM: INITIAL TECHNOLOGY DOWNSELECTION PLAN A-1

B. ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM DOWNSELECT PROJECT TEAM B-1

C. ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNICAL REQUIRMENTS FOR USER SCENARIOS C-1

C. ADDITIONAL INFORMATION ON MILITARY EXPOSURE GUIDELINES D-1

E. POSSIBLE ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNOLOGIES E-1

F. EXAMPLE OF AN ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNOLOGY FACT SHEET F-1

G. ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM DOWNSELECT STRAWMAN MODEL GOALS AND MEASURES G-1

H. EVALUATION METHOD FOR ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM HIGH-LEVEL QUALITATIVE MODEL H-1

I. HIGH-LEVEL QUALITATIVE MODEL AND ASSESSMENT/RESULTS FOR ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNOLOGIES I-1

J. INFORMATION ON SELECTING TRAINING/TEST CHEMICALS AND LIST OF TRAINING/TEST CHEMICALS J-1

K. TEST PLAN TO GATHER NEEDED DATA: TOXICITY SENSOR TESTING TASK K-1

INITIAL TECHNOLOGY ASSESSMENT FOR THE ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM

1. OVERVIEW

1.1 Introduction.

The U.S. Army Center for Environmental Health Research (USACEHR), with support from Army client organizations and funding from Army Science and Technology Objective (STO) IV.ME.2004.03, is developing an Environmental Sentinel Biomonitor (ESB) system to provide rapid toxicity identification for a broad spectrum of chemicals in water. A critical initial phase of the STO is to test and evaluate toxicity sensor technologies (also called ESB system technologies). Because there are a number of potentially feasible technologies that could meet the goals of the ESB program, a downselect will be performed to evaluate these technologies and select the most promising technologies for further development as part of an ESB system.

1.2 Background.

The focus of the ESB system is to detect toxicity associated with non-militarized chemicals (i.e., toxic industrial chemicals [TICs] and toxic industrial materials [TIMs]) in water.

1.2.1 Problem Being Addressed/Problem Definition.

Deployed U.S. forces face the possibility of drinking water exposed to a wide range of toxic industrial or agricultural chemicals as a result of normal use (e.g., farm run-off), damaged infrastructures, accidental spills, or deliberate chemical contamination of water. Unfortunately, rapid detection capabilities for toxic chemicals in water are limited and may not provide sufficient warning of developing toxic hazards.

1.2.2 How the ESB System Addresses the Problem.

The ESB system monitors responses of biological components (e.g., cells, tissues, or whole organisms) exposed to water and provides rapid responses/warnings should toxic conditions develop. Cell-based sensors are becoming available that integrate biological systems with electronic monitoring, facilitating rapid response to developing toxicity in water using very small systems.

1.2.3 ESB Program Objectives and Goals.

The ESB program will incorporate current toxicity sensor technologies into an ESB system having size, weight, and logistical characteristics suitable for a range of Army requirements that will complement current chemical monitoring systems and provide rapid toxicity identification for a broad spectrum of chemicals in water. The optimal system may be a complementary set of toxicity sensors, which would provide the following:

- Rapid response. Required response times may range from a few minutes to an hour depending upon the particular Army use scenario.

- Sensitivity. One ESB system technology may not adequately detect all TICs/TIMs. The evaluation will consider not only which toxicity sensor provides the best overall response to the test chemicals, but also which set of sensors can complement each other by filling gaps in toxicity response for individual sensors as well as providing mutual confirmation of a toxic response.

1.2.4 Desire to Leverage with Other Technologies.

The ESB system development should be integrated with other Army- and DoD-related research and development activities for water analysis. For example, ESB system platform development could leverage work that has already been performed on the Joint Service Agent Water Monitor, particularly in the areas of sample preparation and delivery, sensor platform configuration, and data analysis and display.

2. FY04 ESB SYSTEM INITIAL TECHNOLOGY ASSESSMENT RESULTS

The downselection plan employs a logical, structured decision analysis process, and the results and rationale are documented so that final recommendations can be readily explained and defended (see Appendix A). The results of the downselection evaluation process completed to-date are described below.

2.1 Study/Evaluation Team Formed.

An evaluation team was formed in the fall of 2003 to conduct the downselect. The team is led by Dr. William van der Schalie (USACEHR) and Dr. Thomas Gargan II (GEO-CENTERS, Inc.), with support from the Decision Analysis Team (DAT) of the Edgewood Chemical and Biological Center. The evaluation team is made up of Army user representatives (members of an Integrated Product Team) and technical experts from collateral organizations and academia (see Appendix B for a list of team members and their roles).

The user representative's purpose is to articulate the situations/scenarios (e.g., a small team of special operation forces operating in the hills of Afghanistan) for all possible Army users of the ESB system technology. They know what the end-user needs are because they have the same experiences and knowledge and/or access to others that have this knowledge and experience. The user representatives also define the technical requirements for the ESB system technology for each scenario (e.g., in the small team of special operation forces scenario, the ESB system technology must operate in water temperatures between 0-60 degrees Celsius). Their final task is to help develop the downselection evaluation model. They do this jointly with the technical experts. Although the user representatives have primary responsibility for model development, the technical expert's input is important because they provide input on the technical feasibility of criteria and measures for the model.

The technical expert's purpose is to be knowledgeable about the ESB system technologies, develop any needed preliminary models and help develop the final downselection evaluation model, and then assess the technologies using the preliminary model, if needed, and the final downselection evaluation model.

2.2 ESB System Use Concepts/Applications.

Several Army applications for an ESB system were identified early in the STO program process. These include:

- Use in conjunction with the Tactical Water Purification System (TWPS), including evaluation of water pre- and post-treatment and supporting decisions regarding the suitability of using a freshwater bypass system. The ESB system is consistent with a recommendation in the Pre-Planned Product Improvement section of the TWPS Operational Requirements Document.

- Testing water produced by on-board water generation equipment in manned ground vehicles being developed under the Future Combat System program.

- Use by Preventative Medicine personnel to evaluate potable water quality in a variety of situations.

- Use at Army installations, both domestically and overseas, to evaluate source and drinking water quality.

The user representatives reviewed the above Army applications and identified others. They then grouped and analyzed these applications to form the fewest number of scenarios that would represent all identified Army applications. This was done through an iterative process, and resulted in four basic scenarios to represent the situations in which the Army operates. Two of the four basic scenarios were further subdivided by type of water tested and collection method to create two more scenarios, for a total of six.

The type of water tested could either be raw (i.e., water that has not been treated with any mechanism [e.g., filters] or chemicals [e.g., chlorine]) or treated (e.g., water treated with a TWPS, hand-held water treatment device, or chemicals).

There are two types of collection methods. In the "continuous flow" method, for example, the water must be tested as the water flows inside a pipeline from one point to the next. The ESB system using the continuous flow method must constantly monitor the water for contamination. The results of the tests need to be available in near real-time in order to prevent use or contamination further along in the distribution system. The "grab sample" method is defined as procuring water from a source and then testing it off-line. The water needs to be collected and tested before it is used, with maximum response times ranging between a few minutes to an hour.

2.2.1 Six Scenarios Considered.

2.2.1.1 Individual/Small Team Scenario.

This scenario represents one soldier or a small team of soldiers operating remotely away from a fixed or field/contingency base (e.g., a small team of soldiers operating remotely in the hills of Afghanistan – soldiers are flown in and out of this remote location every 14 days; they must procure local water each day).

The ESB system provides the individual soldier or small team the ability to test a grab sample of raw or treated water in the field for the presence of toxic chemicals that could cause acute health effects and degrade battlefield readiness/performance. The soldier could test raw water from a surface water source prior to disinfecting and drinking it or test water that has been treated using a hand-held water treatment device. This gives the individual soldier an enhanced capability to check the quality of his personally acquired/produced water before consuming it. The ESB system would be used in emergency situations when the soldier does not have access to a doctrinally treated and/or distributed water supply and must acquire, produce, or purify their own water (e.g., initial entry, remote site, and special operation forces operations).

2.2.1.2 Future Combat System - Manned Ground Vehicle Scenario.

This scenario is similar to the individual/small team scenario. Instead of an individual operating with only what they can carry or procure locally (e.g., local untreated water), the soldiers are operating with a vehicle. This vehicle has the ability to generate potable water through a process that starts with capturing the emissions of the vehicle. The purpose of the ESB system is to independently verify the vehicle is producing potable water.

2.2.1.3 Field/Contingency Scenarios.

These two scenarios (differing only in the type of water tested and collection method) represent a field/contingency operation where more than a small team of soldiers is living and working, for more than 15 days, and their water is procured from a questionable source (quality/safety of water is not known). Normally the source water would be treated using a Reverse Osmosis Water Purification Unit (ROWPU) or TWPS.

2.2.1.3.1 Grab Sample to Test Raw or Treated Water Scenario.

A reconnaissance team, in its mission to find a suitable source of water for the ROWPU or TWPS units, must test existing water sources in the area of the field/contingency base. Also, Preventative Medicine personnel need to periodically check for TICs/TIMs in storage tanks and distribution systems. The objective is to catch accidental or intentional spills, discharges or contamination.

2.2.1.3.2 Continuous Flow Sample to Test Treated Water Scenario.

Quartermaster personnel could use a continuous flow ESB system to monitor both water that is the source for their ROWPU or TWPS as well as the water produced by these units/systems.

2.2.1.4 Fixed-Base Scenarios.

These two scenarios (differing only by type of water tested and collection method) represent operations at an existing base either within the United States or overseas. In these scenarios, the soldiers obtain their drinking water from water treatment plants that meet U.S. quality standards (potable water is not being created with a ROWPU or TWPS). The water treatment plant could be located either on-base or off-base, with the water piped in. The population will be less homogeneous than the other scenarios because dependents will be using the water supply.

2.2.1.4.1 Grab Sample to Test Raw or Treated Water Scenario.

Public Works, Preventative Medicine, and possibly Security personnel must perform spot checks of water system assets (e.g., treatment plant effluent, individual storage tanks, distribution system) for the presence of TICs/TIMs at levels that could cause harmful health effects in installation populations.

2.2.1.4.2 Continuous Flow Sample to Test Raw or Treated Water Scenario.

Installation Public Works personnel must monitor continuously for the introduction of TICs/TIMs into the base's water supply system in order to prevent or minimize problems.

2.3 Technical Requirements Defined.

The user representatives developed technical requirements for the six scenarios (section 2.2.1). These requirements define in detail the performance specifications for each ESB system technology. The ESB system technical requirements are made up of 26 technical requirement questions (covering eight evaluation categories) and their answers (see Appendix C). The purpose of this requirements document is to provide more specific information for the individual scenarios and to help the expert panel know what information must be collected (though research or additional testing) for the downselection evaluation. The document is not intended to be the final design specification for an ESB system. The user representatives noted both threshold/minimum requirements and objective ("give me everything I want") requirements. The next section discusses what sensitivity the ESB system needs to identify toxicity associated with industrial chemicals.

Chose and Defined Target Detection Range.

The range the ESB system technology must detect TICs/TIMs within (upper and lower limit) must be specified before the technologies can be evaluated.

The users agreed the threshold requirement for the ESB system technologies is to detect TICs/TIMs between the short-term Military Exposure Guidelines (MEGs) level (standard for a soldier drinking 15 liters/day for 14 days, see Appendix D for additional information about MEGs) and the human lethal concentration (HLC), which assumes consumption of 15 L in a one day period by a 70 kg soldier. The ESB project will not focus on detecting TICs/TIMs at more sensitive levels (e.g., long-term MEG, EPA – where effects are chronic) until more promising technology becomes available. Until then operations will use existing analytical chemistry tools to detect TICs/TIMs at chronic effect levels.

The user representatives noted that sensors should detect toxicity closer to the short-term MEG level than the HLC level and that the minimum detection level must be below the HLC. Finally, user representatives stated that detecting toxicity below the short-term MEG level is not desirable because this would result in false positive readings.

2.4 Candidate ESB System Technologies Identified and Partially Described.

After extensive research, 38 possible ESB system technologies were identified (listed in Appendix E) for further research and evaluation. Fact sheets were created for each technology (see Appendix F for an example) and presented to all expert panel members. The information within the fact sheets encompasses performance, operational, and logistical characteristics.

2.5 Expert Team Determines Additional Testing is Needed.

The expert team determined that there was insufficient toxicity response information for a thorough comparative evalution of the ESB system technologies.

2.6 Interim Downselection Process Completed.

The six scenarios (section 2.2.1) and technical requirements (section 2.3) are the foundational information needed to develop the downselection evaluation model. Because critical data about the technologies were not available, the final downselection evaluation model could not be completed. An additional step, a qualitative evaluation, was added to the process to reduce the number of technologies to test (based on what could be funded) to generate the critical data.

2.6.1 Overview Description of Downselection Evaluation Model Components and How Model Is Being Developed.

Evaluation criteria are a core component of the model. The criteria are comprised of definitions, performance scales, and weights. Criteria are quantitative (e.g., response time) or

qualitative (e.g., ease of use) in nature. The criteria are structured as a hierarchical model and are at a level that permits discrimination between the different technologies. Higher level criteria categories, referred to as goals, include factors such as effectiveness/performance, operational, logistics, and safety, health, and environment. Specific sub-categories of criteria, referred to as measures, are developed to provide the degree of discrimination needed for the technology evaluation. Each measure has an associated performance scale to be used to score the technologies. These scales are expressed in natural units such as minutes, or are constructed scales. Utility curves are developed for each performance scale. The final step in model development is to weight the criteria. A number of techniques are available to help the evaluation team determine the relative priorities of the goals and measures.

2.6.2 Development To Date of Downselection Evaluation Model.

Significant progress was made in developing the downselection evaluation model during a meeting with user representatives and the expert panel (see Appendix G). The final model will be completed after the required data for ESB system technologies is generated.

A strawman (initial draft, a starting point) evaluation model was developed initially by the DAT. The model was then reviewed and refined by the evaluation team after the scenario development (made up of applications and use concepts) and all needed data for the ESB system technologies become known. The user representatives have primary responsibility for model development; however the technical experts provide input on technical feasibility. Although the goal is to derive one evaluation model that represents all requirements, multiple models may be needed in order to properly evaluate all scenarios.

2.6.3 Expert Panel Evaluated Technologies Using a High-Level Qualitative Model.

As stated in the previous section, the final downselect for the ESB system technologies could not be done without additional data. Because of funding and time constraints, it was not possible to gather the needed data for all 38 technologies. A high-level qualitative model was created to choose the most promising ESB system technologies to take into the lab for further testing.

The evaluation method used a two-step process. The first step in the process was to determine whether a technology could meet all threshold requirements of the Army STO. Twenty-three of the 38 technologies were eliminated by this step. In the final step the 15 remaining technologies were evaluated against four high level criteria and a final ranking was generated. The criteria were: 1) sensitivity response and/or provides unique information, 2) interferences, 3) reliable/repeatable, and 4) rapidity of response (see Appendix H for details on the evaluation method and Appendix I for the results).

Expert Panel Chose Nine Technologies for Testing.

The following ESB system technologies were determined to be the most promising (1 is most promising, 14 least promising) by the expert panel. The technologies in bold text are being funded for additional testing, notes are provided why others were not funded.

1. **Eclox** or Aquanox (expert panel only wanted to test one of these technologies because they are almost the same in performance and the way they work [i.e., mechanisms, modes of action], with additional research Eclox was determined to be slightly better so Aquanox was dropped from further consideration).

2. **Microtox**

3. **Mitoscan**

4. **ToxScreen**

5. ***Sinorhizobium meliloti* Assay**

6. **SOS Cytosensor**

7. **Portable Neuronal Microelectrode Array**

8. **Portable Cell-based Biosensor** (owner of technology unable to participate in further testing at this time, may be able to test later in FY05).

9. **Cellsense** (European-based technology, owner of technology not responsive to request to participate in further testing).

10. **Amtox** (extremely costly to test; may consider testing later if funding becomes available).

11. **Toxi-chromo Test**

12. **Fluotox** (European-based technology, owner of technology not responsive to request to participate in further testing).

13. **ArrayScan** HCS System (funding not available).

14. **Epithelial Cell Toxicity Sensor** (testing is provided at no additional cost).

2.7 Selected Training/Test Chemical Set, Developed Test Plan, and Started Testing Nine Technologies.

Prior to finalizing a test plan to collect the data for the nine technologies, the expert panel, with input from the Army user representatives, identified and selected a set of training/test chemicals. These chemicals are being used to form a common basis for comparison of test results for the nine ESB systems technologies that are being further tested and will used to generate new toxicity data. Twelve chemicals were selected by the expert panel to be the training/test chemical set (see Appendix J for a more detailed discussion on the selection of test chemicals and the list of chemicals).

To acquire toxicity response data, an experimental design and analysis plan was developed (see Appendix K for additional information on the test plan) and a contract was issued to Battelle Memorial Institute in late FY04 to collect this data. A statistician provided peer review of the experimental design. Testing should be completed during the 2nd quarter of FY05.

3. FY05 PLANS TO COMPLETE THE ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNOLOGY DOWNSELECTION PROCESS.

In FY04 the groundwork for a successful downselection in FY05 was completed. By collecting the detailed data needed for each of the nine ESB system technologies early in FY05 it should be possible to complete the downselect before mid-year. The final steps in the downselection process are described next.

3.1 Complete Data Collection for Nine "Finalist" Technologies.

The testing that started in FY04 should be completed by the middle of the 2nd quarter in FY05. The testing process is being managed through a contract with Battelle Memorial Institute.

3.2 Complete Evaluation Model.

After the test data becomes available, the DAT will refine the initial downselection evaluation model and present it to the user representatives and expert panel. The team will then finalize the model jointly at a meeting.

3.3 Evaluate Nine Technologies.

The expert panel will complete the downselect evaluation by assessing each technology against each criterion in the evaluation model(s). For this meeting, primary input will come from the technical experts. The assessment process will be facilitated by the DAT, and final scoring will be based on group consensus. This step should be complete by the end of the 2nd quarter in FY05.

3.4 Complete Analysis and Produce Final Report.

The analysis will be performed after the nine ESB system technologies are evaluated using the downselect evaluation model. The DAT will analyze the information generated during the technology assessment phase as described below.

First, an overall score for each technology will be generated based on individual criterion scores and the weight given to each criterion. Detailed analysis will be conducted to summarize why the technologies scored as they did, to include identifying significant strengths and weaknesses for each technology relative to the criteria. This will be important in determining whether there are synergies between the technologies (it is unlikely one technology will satisfy all requirements). It will also allow researchers and/or users to choose the technology that best fits their needs (it's possible the technology with the highest overall ranking may not be the ideal technology to use for a particular user because of their unique needs). Analysis will also be conducted to identify areas of technical challenge to help focus future research and development efforts. Finally, sensitivity analysis will be performed to determine the impact of model variations (e.g., alternative weighting schemes or scoring uncertainties) on the results.

The DAT will produce a final report similar in scope and detail to this initial report that incorporates the work completed in FY05.

APPENDIX A

ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM: INITIAL TECHNOLOGY DOWNSLECTION PLAN

I. Introduction. Deployed US forces face the possibility of drinking water exposures to a wide range of toxic industrial or agricultural chemicals as a result of damaged infrastructures, accidental spills, or deliberate chemical contamination of water. Unfortunately, rapid detection capabilities for toxic chemicals in water are limited and may not provide sufficient warning of developing toxic hazards. The U.S. Army Center for Environmental Health Research (USACEHR) is developing an ESB system to complement chemical monitoring systems and provide rapid toxicity identification for a broad spectrum of chemicals in water.

ESB systems monitor responses of biological components (i.e., cells, tissues, or whole organisms) exposed to water and to provide rapid responses should toxic conditions develop. Cell- and tissue-based sensors are becoming available that integrate biological systems with electronic monitoring, facilitating rapid response to developing toxicity in water using very small systems. With support from Army client organizations and funding from a new Army Science and Technology Objective (STO), USACEHR will incorporate these toxicity sensors into an ESB system having size, weight, and logistical requirements suitable for deployment. This paper provides an overview of the toxicity sensor downselection process and identifies associated technical issues.

II. ESB Technology Downselection. A critical initial phase of the STO is to test and evaluate toxicity sensors. Because there are a number of potentially feasible technologies that could meet the goal of the ESB program, a downselect will be performed to evaluate these technologies and select the most promising technologies for test and evaluation. The downselect will employ a logical, structured decision analysis process, and the results and rationale will be documented so that final recommendations can be explained and defended. This downselect process will be comprised of several distinct parts/phases, as described below.

A. Select study/evaluation team. An evaluation team will be formed to conduct the downselect. The team will be led by Dr. William van der Schalie (USACEHR) and Dr. Thomas Gargan II (GEO-CENTERS, Inc.), with support from the Decision Analysis Team (DAT) of the Edgewood Chemical and Biological Center. The evaluation team will be made up of Army user representatives (members of an Integrated Product Team - IPT) and technical experts from collateral organizations and academia.

B. Determine use concepts/applications. Army applications for an ESB system have been identified as part of the STO program definition. Examples include:

- Use in conjunction with the Tactical Water Purification System (TWPS), including evaluation of water pre- and post-treatment and supporting decisions regarding the suitability of using a freshwater bypass system. The ESB system is consistent with a recommendation in the Pre-Planned Product Improvement section of the TWPS Operational Requirements Document.

- Testing water produced by on-board water generation equipment in manned ground vehicles being developed under the Future Combat Systems (FCS) program.

- Use by Preventive Medicine personnel to evaluate potable water quality in a variety of situations.

- Use at Army installations, both domestically and overseas, to evaluate source and drinking water quality.

IPT members will be responsible for identifying applications for the ESB system and defining in detail how the system might be used (Conditions of Operation - CONOPS). This information will be important to help define desired system characteristics.

C. Identify and describe candidate technologies. Toxicity sensor technologies that have potential to meet ESB program needs will be identified and described. The information that will be described encompasses performance, operational, and logistical characteristics. The technology information will be documented in a fact sheet format. This information will be provided to the study team participants prior to evaluation model development, so everyone has an understanding of the range of technologies that will be evaluated.

D. Develop evaluation model (criteria, definitions, performance scales, weights). The applications, CONOPS, and requirements will be used as the foundation to derive evaluation criteria for the downselect. The criteria will be comprised of definitions, performance scales, and weights. Criteria can be quantitative (e.g., response time) or qualitative (e.g., ease of use) in nature. The criteria will be structured as a hierarchical model and will be at a level that will permit discrimination between the different technologies. Higher level criteria categories, referred to as goals, include factors such as effectiveness/performance, operational, logistics, and safety, health, and environment. Specific sub-categories of criteria, referred to as measures, will be developed to provide the degree of discrimination needed for the technology evaluation. Each measure will have an associated performance scale to be used to score the technologies. These scales can be expressed in natural units such as time, or may be constructed scales. Utility curves will also be developed for each performance scale. The final step in model development will be to weight the criteria. A number of techniques are available to help the evaluation team determine the relative priorities of the goals and measures.

A strawman evaluation model will be developed initially. This model will be reviewed and refined by the evaluation team during the initial evaluation team meeting after the use concepts are developed. Again, the IPT will have primary responsibility for model development, although the technical experts will provide input on technical feasibility.
Although the goal is to derive one evaluation model that represents all requirements, multiple models may be needed in order to properly evaluate all scenarios.

E. Evaluate technologies. The evaluation team will convene to assess the technologies against the evaluation model(s). For this meeting, primary input will come from the

technical experts. The assessment process will be facilitated by the DAT, and final scoring will be based on group consensus.

F. Perform analysis and report results. The DAT will analyze the information generated during the technology assessment phase. Logical Decisions for Windows (LDW), a decision analysis software tool, will be used to support the analysis.

The analysis phase will consist of a number of steps. First, an overall score for each technology will be generated based on individual criterion scores and the weight given to each criterion. Detailed analysis will be conducted to summarize why the technologies scored as they did, to include identifying significant strengths and weaknesses for each technology relative to the criteria. This will be important in determining whether there are synergies between the technologies (it is unlikely one technology will satisfy all requirements). It will also allow researchers and/or users to choose the technology that best fits their needs (it's possible the technology with the highest overall ranking may not be the ideal technology to use for a particular user because of their unique needs). Analysis will also be conducted to identify areas of technical challenge to help focus future research and development efforts. Finally, sensitivity analysis will be performed to determine the impact of model variations (e.g., alternative weighting schemes or scoring uncertainties) on the results.

A report of the study will be prepared by the DAT.

III. ESB Downselection Issues. There are a number of key issues relevant to toxicity sensor selection and evaluation. It is hoped that this paper will stimulate discussion that will help further define the developmental process for the ESB system. Key questions include:

- What toxicity benchmarks should be used to evaluate toxicity sensor responses to chemicals?

- What kind of (and how many) test chemicals should be used to evaluate toxicity sensor performance?

- How should toxicity sensor performance be evaluated?

- Can chemical monitoring technologies be leveraged with the ESB system operation?

A. Toxicity benchmarks. To select an appropriate toxicity benchmark for evaluating ESB toxicity sensors, the toxicity target must first be specified. As defined in the original STO proposal, the desired STO product is a system that will "... provide rapid identification of acute toxic hazards in water". The goal is to quickly respond to toxic exposures that could affect a soldier's health and performance in the field.

One possible frame of reference for evaluating toxicity sensor performance is provided by the Military Exposure Guidelines (MEGs; USACHPPM, 2001). MEGs are provided for

water (and other media) to estimate a level "... above which certain types of health effects may begin to occur in individuals amongst the exposed population". MEGs are "... designed to indicate 'thresholds' for minimal to no adverse health effects" and are considered "... protective against any significant non-cancer effects". MEG exposure scenarios for water are appropriate for a deployed military operation, i.e., exposure lasting either 5 or 14 days, with water consumption of either 5 or 15 L/day. The exposed population is defined to include "... relatively healthy and fit male and non-pregnant female adults", 18 to 55 years old with and average weight of 70 kg. It is important to note that while MEGs are not enforceable military standards, the MEGs are considered guideline concentrations for identifying and ranking Occupational and Environmental Health (OEH) risks. MEGs have been established for about 170 chemicals. The National Research Council's Board on Environmental Studies and Toxicology is conducting a review of the MEGs in a project entitled "Review of the Army's Technical Guidance Documents on Assessing Toxicological Risks from Exposures to Chemicals"; a report is currently in preparation.

MEGs provide a reference point above which adverse effects may be expected after a field-relevant period of exposure and may serve as lower thresholds for toxicity sensor responses. That is, responses at concentrations below the MEGs indicate toxic effects that may not be relevant to acute human health impairments.

What then should be an upper threshold for toxicity sensor responses? One possibility is the human oral lethal dose, which has been compiled for a number of chemicals by the Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) program. The MEIC program evaluated the use of 67 *in vitro* cytotoxicity tests for predicting acute systemic toxicity in humans for a set of 50 test chemicals representing a wide range of modes of toxic action (Clemedson *et al.*, 1998; Ekwall *et al.*, 2000). The MEIC analysis evaluated the relationship between cytotoxicity assay results and human lethal blood concentrations and the ability of rodent LD_{50}s to predict human oral lethal doses. Although cytotoxicity assay results are logically compared with lethal blood concentrations rather than lethal dose (i.e., without adding the compounding factors of intervening uptake, metabolism, and depuration), ESB toxicity sensors must evaluate drinking water suitability directly based on evaluation of the water to which the sensors are exposed. Thus, for the ESB project, human oral lethal doses may be a more appropriate measure of the upper response level for a toxicity sensor than human lethal blood concentrations.

Figure 1 illustrates one possible approach for evaluating the suitability of a toxicity sensor for identifying the presence of toxic chemicals in drinking water. Shown is the relationship between MEGs, corresponding human lethal doses, and responses of one human cell cytotoxicity assay (Chang liver cell) for the 14 chemicals having both MEGs and MEIC study data. The chemicals are arranged in order of increasing human lethal dose. For most chemicals, there is a two to four log difference between the MEG and the corresponding human lethal dose. A notable exception is cyanide, for which the difference is a factor much less than one log. Only six of the 14 IC_{50}s (concentrations causing a change in morphology in 50% of exposed cells) fall between the acute lethal human value and the corresponding MEG (Figure 1). However, if technology were available to

concentrate a chemical within a water sample the relationship between the chemical IC_{50} value and its corresponding lethal human value and MEG would change dramatically (Figure 2). In this case, a factor of 10 concentration of a tested water sample containing the chemicals would put the IC_{50}s for all but one chemical (cyanide) within the MEG-acute lethal range (Figure 2).

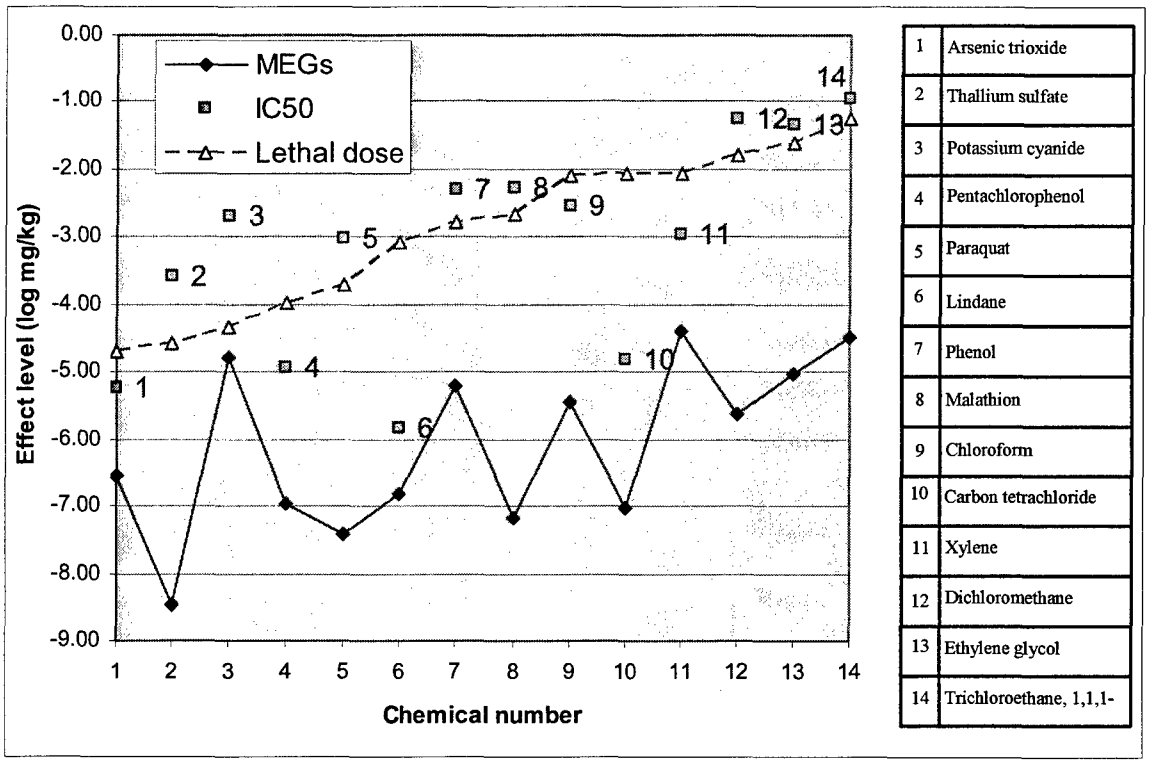


Figure 1. Human cell line (Chang liver cells) 24-h IC_{50}s for 14 chemicals (Clemedson *et al.*, 1996) compared with corresponding acute human lethal concentrations (Ekwall *et al.*, 1998) and Military Exposure Guideline (MEG) levels (USACHPPM, 2001). One day of exposure to a 14-day MEG was converted to dose assuming 5 L/day water consumption by a 70 kg adult.

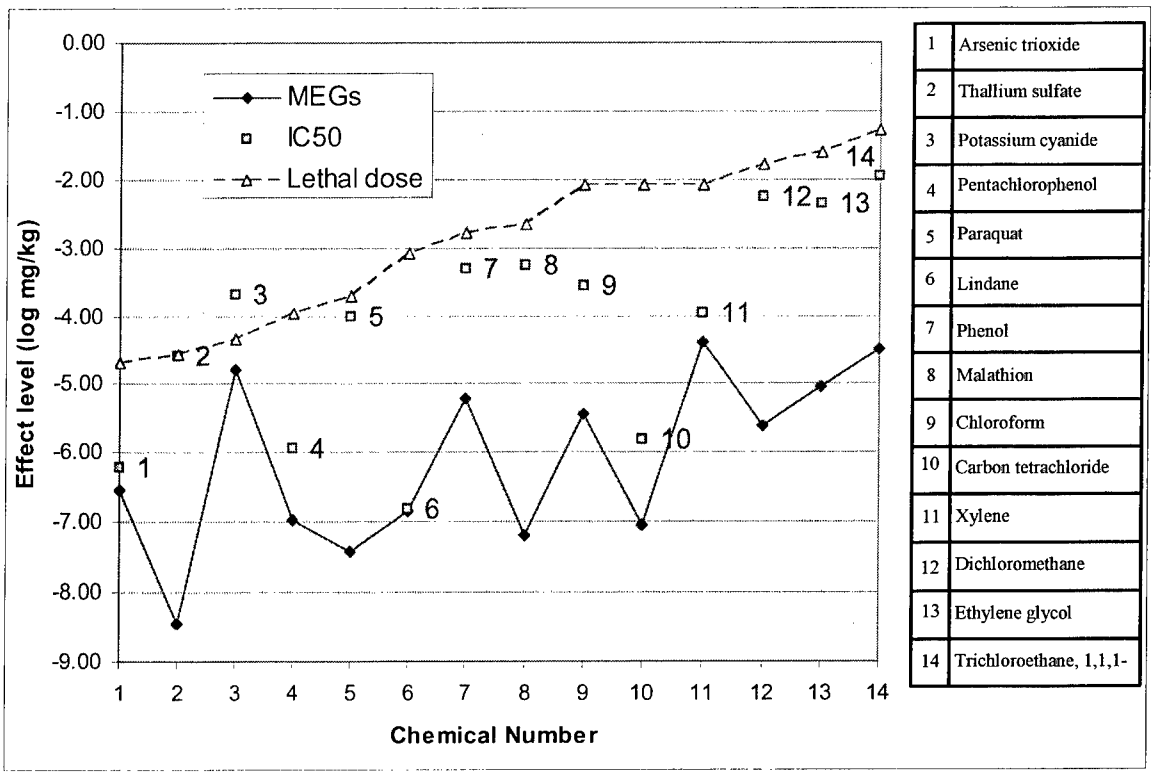


Figure 2. Effect of a hypothetical ten-fold concentration of water samples on Figure 1 IC_{50} data. Human cell line (Chang liver cells) 24-h IC_{50}s (divided by 10) for 14 chemicals (Clemedson *et al.*, 1996) compared with corresponding acute human lethal concentrations (Ekwall *et al.*, 1998) and Military Exposure Guideline (MEG) levels (USACHPPM, 2001). One day of exposure to a 14-day MEG was converted to dose assuming 5 L/day water consumption by a 70 kg adult.

B. Selection of test chemicals. Considering the thousands of toxic industrial and agro-chemicals, an appropriate evaluation of toxicity sensor performance requires careful initial selection of the chemicals to be evaluated. Some possible criteria for selecting test chemicals include:

- Threat chemicals. Several organizations (U.S. Environmental Protection Agency (EPA), U.S. Army Research Development and Engineering Center (ARDEC)) and publications (Garland, 1991; Hickman, 1999; Clark and Deininger, 2000) have developed lists of threat chemicals for water supplies. Typically, the higher priority chemicals are highly toxic, soluble, persistent in water, and available in the region of concern. Chemicals high on these lists should be given priority as test chemicals for toxicity sensors.

- Mode of toxic action. Some chemicals can be grouped together because they cause similar effects on organisms. Examples include organophosphorus and carbamate insecticides (which cause acetylcholinesterase inhibition) and many neutral organic chemicals such as industrial solvents (which cause narcosis). To the extent that chemicals can be grouped in this way, it should be possible to select one or two representative chemicals from the group for

testing to establish whether a toxicity sensor will respond to chemicals exhibiting the same general mode of toxic action.

- Chemical structural classes. Chemicals with certain structural, functional, or other similarities are frequently grouped together for testing purposes, such as heavy metals, algal toxins, or herbicides. Since chemicals within these groups may have distinctly different modes of toxic action, it is best to emphasize mode of toxic action categories in the selection of test chemicals. The MEIC chemical list provides a broad range of both modes of toxic action and chemical structural classes (Clemedson *et al.*, 1996, 1998).

- Interfering chemicals or water quality conditions. Interfering chemicals include substances commonly found in raw or treated water that may cause toxicity sensor responses at levels far below concentrations affecting humans, such as residual chlorine, copper, or ammonia. In addition, acceptable ranges of common water quality conditions, such as temperature, dissolved oxygen, conductivity, pH, turbidity, and microbial concentrations need to be defined for toxicity sensors to help minimize "false positive" responses.

Given the broad range of potential test substances, a compromise must be reached between minimizing the number of chemicals to conserve resources and expanding the number to better define toxicity sensor response characteristics. One possible solution is the use of a tiered approach – conduct an initial screening of several toxicity sensor technologies with a few chemicals, followed by more thorough evaluation of technologies showing the most promise. Consideration should be given to identifying a complementary set of toxicity sensors with regard to:

- Rapid response. A very rapid (e.g., response in a few minutes) evaluation with an inexpensive test that may be prone to false positives could be followed by a more reliable but slower (e.g., response in an hour) toxicity test for confirmation.

- Sensitivity. One toxicity sensor type may not adequately cover all chemicals. Sensor evaluation should consider not only which toxicity sensor provides the best overall response to the test chemicals, but which set of sensors can complement each other by filling gaps in toxicity response for individual sensors as well as providing mutual confirmation of a toxic response.

C. Defining toxicity sensor performance. When it is time to evaluate the response characteristics of a toxicity sensor to selected test chemicals, the issue of defining a response threshold (= cutoff point) must be addressed. Receiver Operator Characteristic (ROC) curves, a type of statistical decision theory that have recently become a very useful tool in medical-decision making. A web-based ROC curve tutorial is available (http://www.anaesthetist.com/mnm/stats/roc/ (Click the Refresh button if the page does not immediately open).

ROC curves are a graphic method of showing the true positive rate (sensitivity) and the false positive rate (1-specificity) at each possible cutoff point (= threshold) of the test results. The major advantage of ROC curve analysis is in defining the "cutoff" between "positive" and "negative" values. ROC curves are useful in accessing the discriminatory power of diagnostic tests and evaluating sensor performance in a broad range of fields. The following URLs show some recent uses of ROC curve analysis to evaluate sediment quality guidelines for metals (http://www.hsph.harvard.edu/shinelab/ROCpresentation.pdf) and beach water quality indicator variables (http://aem.asm.org/cgi/reprint/69/11/6405.pdf).

The ESB system may utilize various types of ROC curve analysis, namely, in the diagnostic accuracy of a toxicity sensor to identify a toxicant at various concentrations, comparing tests repeated on different occasions, and comparing ability of different toxicity sensors to detect a toxicant. The ESB program is planning on using a statistician to help in the experimental design and the analysis of the toxicity sensor performance.

D. Technology leveraging. ESB system development must be integrated with other Army- and DoD-related research and development activities for water analysis. As a matter of principle, ESB system platform development should leverage work that has already been performed on the Joint Service Agent Water Monitor (JSAWM), particularly in the areas of sample preparation and delivery, sensor platform configuration, and data analysis and display. Where possible, similar designs and approaches should be employed so that the ESB system and the JSAWM are compatible. While ESB system development is focused on toxicity sensors, the availability of simple, rapid screening technologies for a wide range of toxic industrial materials should be tracked and incorporated in the ESB approach where possible to provide confirmatory evidence of a chemical etiology for a toxicity sensor response. Similarly, although pathogen identification is not an objective of the ESB STO, possible dual use technologies for chemical toxicity and pathogen identification should be considered. One example is the "CANARY cells" (Cellular Analysis and Notification of Antigen Risks and Yields; Rider *et al.*, 2003), which are mammalian lymphocytes specifically designed for pathogen identification but which might have application for toxicity identification as well.

IV. References

Clark RM, Deininger RA. 2000. Protecting the nation's critical infrastructure: The vulnerability of U.S. water supply systems. *Journal of Contingencies and Crisis Management* 8:73-80.

Clemedson C, McFarlane-Abdulla E, Andersson M, Barile FA, *et al.* 1996. MEIC evaluation of acute systemic toxicity Part II. *In vitro* results from 68 toxicity assays used to test the first 30 reference chemicals and a comparative cytotoxicity analysis. *ATLA* 24:273-311.

Clemedson C, Andersson M, Aoki Y, Barile FA, *et al.* 1998. MEIC evaluation of acute systemic toxicity Part IV. *In vitro* results from 67 toxicity assays used to test reference chemicals 31-50 and a comparative cytotoxicity analysis. *ATLA* 26:131-183.

Ekwall B, Clemedson C, Crafoord B, Ekwall B, Hallander S, Walnut E, Bondesson I. 1998. MEIC evaluation of acute systemic toxicity Part V. Rodent and human toxicity data for the 50 reference chemicals. *ATLA* 26:571-616.

Ekwall B, Ekwall B, Sjostrom M. 2000. MEIC evaluation of acute systemic toxicity Part VIII. Multivariate partial least squares evaluation, including the selection of a battery of cell line tests with a good prediction of human acute lethal peak blood concentrations for 50 chemicals. *ATLA* 28:201-234.

Garland JG. 1991. Water Vulnerability Assessments. AL-TR-1991-0049. Armstrong Laboratory, Occupational and Environmental Health Directorate, Brooks Air Force Base, TX.

Hickman, DC. 1999. A Chemical and Biological Warfare Threat: USAF Water Systems at Risk. Counterproliferation Paper No. 3. USAF Counterproliferation Center, Air War College, Maxwell Air Force Base, AL.

Rider TH, Petrovick MS, Nargi FE, Harper JD, Schwoebel ED, Mathews RH, Blanchard DJ, Bortolin LT, Young YM, Chen J, Hollis MA. 2003. A B cell-based sensor for rapid identification of pathogens. *Science* 301:213-215.

U.S. Army Center for Health Promotion and Preventive Medicine. 2001. Chemical Exposure Guidelines for Deployed Military Personnel. TG-230. U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD.

V. Acronyms

ARDEC	Army Research Development and Engineering Center
CANARY	Cellular Analysis and Notification of Antigen Risks and Yields
CONOPS	Conditions of Operation
DAT	Decision Analysis Team
DoD	Department of Defense
EPA	US Environmental Protection Agency
ESB	Environmental Sentinel Biomonitor
FCS	Future Combat Systems
IC	Inhibitory Concentration
IPT	Integrated Product Team
JSAWM	Joint Service Agent Water Monitor
LD	Lethal dose
LDW	Logical Decisions for Windows
MEG	Military Exposure Guidelines
MEIC	Multicenter Evaluation of *In Vitro* Cytotoxicity
OEH	Occupational and Environmental Health
ROC	Receiver Operator Characteristic
STO	Science and Technology Objective
TWPS	Tactical Water Purification System
US	United States
USACEHR	United States Army Center for Environment Health Research
USACHPPM	United States Army Center for Health Promotion and Preventive Medicine
URL	Universal Resource Locator

APPENDIX B

ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM DOWNSELECTION PROJECT TEAM		
First name	**Last Name**	**Affiliation**
Ric	De Leon	Metropolitan Water District of Southern California
Jay	Dusenbury	US Army Research, Development and Engineering Command
Tom	Gargan	U.S. Army Center for Environmental Health Research
Mike	Goode	Edgewood Chemical Biological Center
John	Harbell	Institute for In Vitro Sciences
Wally	Hayes	Harvard School of Public Health
Janet	Jensen	Edgewood Chemical Biological Center
Scott	Kooistra	Edgewood Chemical Biological Center
Joe	Pancrazio	National Institutes of Health (Neurological Disorders and Stroke)
Steve	Richards	U.S. Army Center for Health Promotion and Preventive Medicine
Robert	Ryczak	U.S. Army Center for Health Promotion and Preventive Medicine
Stanley	States	Pittsburgh Water & Sewer Authority
Roy	Thompson	Edgewood Chemical Biological Center
Bill	Van der Schalie	U.S. Army Center for Environmental Health Research
Jeremy	Walker	US Army Research, Development and Engineering Command
John	Walther	Edgewood Chemical Biological Center
Mike	Wright	Boeing, Inc. (Representative for Lead System Integrator, Future Combat Systems – Manned Ground Vehicle program)

Blank

APPENDIX C

ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or maximum requirement (O1 is a higher priority than O2)

#	Category	Tech. Requirements	Scenarios					
			Individual/Small Team	Manned Ground Vehicle	Field/Contingency		Fixed Base	
			Test Raw Water	Test Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
	Collection Method	Continuous flow or grab sample?	Grab	Grab	Grab	Continuous	Grab	Continuous
1	Detection	Does device need to determine a fixed level of toxicity only (e.g., acute or MEG) or give an adjustable/sliding scale reading (e.g., giving a reading between acute and MEG)?	Detect and trigger at a fixed level (Acute[T])).	Detect and trigger at a fixed level (Acute[T])).	Detect and trigger at a fixed level (Acute, MEG-listed TICs for short term consumption [T]). Detect and trigger at two fixed level (Acute & MEG-listed TICs at 1-yr consumption levels [O]).	Detect and trigger at a fixed level (Acute [T]). Detect and trigger at two fixed level (Acute & MEG [O]).	Detect and trigger at a fixed level (Acute [T]). Detect and trigger at three fixed level (Acute, MEG, & EPA [O]) but even better is a sliding scale reading for Acute, MEG, and EPA (O).	Detect and trigger at a fixed level (Acute [T]). Detect and trigger at three fixed level (Acute, MEG, & EPA [O]) but even better is a sliding scale reading for Acute, MEG, and EPA (O).
2	Detection	What toxic industrial chemicals need to be detected? Chemicals that are most important to detect? For example, which are more important the chemicals that effect cognitive functions or gastrointestinal (GI) functions?	CNS/CVS effects w/r to short-term MEG-listed TICs (T) Above + PNS, liver, kidneys (O1), Above + GI tract (O2)	CNS/CVS effects w/r to short-term MEG-listed TICs (T) Above + PNS, liver, kidneys (O1), Above + GI tract (O2)	MEG-listed TICs with CNS/CVS effects (T) Above + PNS, liver, kidneys, GI tract (O)	MEG-listed TICs with CNS/CVS effects (T) Above + PNS, liver, kidneys, GI tract (O)	TICs with CNS/CVS effects (T) Above + PNS, liver, kidney, GI tract (O)	TICs with CNS/CVS effects (T) Above + PNS, liver, kidney, GI tract (O)
3	Detection	What's the level of detection for each chemical?	Acute, i.e. MEG-short term (T)	Acute, i.e. MEG-short term (T)	MEG - short + long term (O)-Acute, i.e., MEG -short term(T)	MEG - short + long term (O)-Acute, i.e., MEG - short term(T)	EPA(O)-MEG (O)-Acute (T)	EPA(O)-MEG (O)-Acute (T)

APPENDIX C

ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or maximum requirement (O1 is a higher priority than O2)

#	Category	Tech. Requirements	Scenarios					
			Individual/Small Team	Manned Ground Vehicle	Field/Contingency		Fixed Base	
			Test Raw Water	Test Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
	Collection Method	Continuous flow or grab sample?	Grab	Grab	Grab	Continuous	Grab	Continuous
4	Environmental Conditions During Test	Range for air temperature, atmospheric pressure/altitude, and humidity level?	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)	1. Operational: 5-95% RH (T)2. Operational: 0C to 45C (T); -32C to 49C (O)3. Storage: 0C to 52C storage (T); -10C to 71C (O)
5	Environmental Conditions During Test	Other water characteristics device must handle (e.g., quantity of total dissolved solids, turbidity, chlorine residual)?	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).	Must function with presence of many dissolved solids up to brackish and turbidity in raw water or chlorine residual (2 ppm (T), 5 ppm (O) with treated water (T).
6	Environmental Conditions During Test	Range for water pH and temperature?	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)	pH: 5-9 (T), 4-10 (O) Temp: 0-49 C (T), 0-60 C (O)

APPENDIX C

ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or maximum requirement (O1 is a higher priority than O2)

#	Category	Tech. Requirements	Scenarios					
			Individual/Small Team	Manned Ground Vehicle	Field/Contingency		Fixed Base	
			Test Raw Water	Test Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
	Collection Method	Continuous flow or grab sample?	Grab	Grab	Grab	Continuous	Grab	Continuous
7	Logistics	Power requirements? Amount? Type (e.g., solar, AC[110, 220], DC [battery])?	Internal power source (T)	External power (T), Internal power source (O)	External power (T), Internal and external power source (O)	External power (T), Internal and external power source (O)	External power (T), Internal and external power source (O)	External power (T), Internal and external power source (O)
8	Logistics	Size and weight of support requirements (consumables, replacement parts, storage)	7 days worth of consumables (15 liters/day per person * 1.5) (T) 14 days (O)	7 days worth of consumables (15 liters/day per person * 1.5) (T) 14 days (O)	1 cu ft/5 lbs (T) 1/2 cu ft/2.5 lbs (O)	2 cu ft/10 lbs (T) 1 cu ft/5 lbs (O)	Minimal concern for installation testing	Minimal concern
9	Physical Characteristics	Controls (dials, switch, knob, etc.) operable in protective and cold weather suits	MOPP (T)	MOPP (T)	MOPP (T)	MOPP (T)	Level A (T)	Level A (T)
10	Physical Characteristics	Display (visible in low light conditions, no sound, remote alarm for continuous monitoring)	Visible in low light conditions, no sound (T)	Visible in low light conditions, no sound (T)	Visible in low light conditions, no sound (O)	Visible in low light conditions, no sound (O). Remote alarm (O)	No requirement	Remote alarm (O)
11	Physical Characteristics	Max. cube, length, and weight?	Credit card size (O). (T) Needs to be researched further.	Max 1 cu ft, 10 lbs (O)	Two cubic feet, 20 lbs (T) One cubic foot, ten lbs (O)	Two cubic feet, 20 lbs (T) One cubic foot, ten lbs (O)	Field device (see Field/Contingency info) or installation level device (6 cu ft/ 40 lbs (T))	Could be as large as a portable lab trailer, as long as can tap pipe continuously (T). 6 cu ft/40 lbs (O)
12	Robustness	Does device still work if it becomes immersed in water or dropped (from 3 feet onto concrete)?	Yes (T)	Yes (T)	Yes (T)	Yes (T)	Yes (O)	Yes (O)

APPENDIX C

ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or maximum requirement (O1 is a higher priority than O2)

#	Category	Tech. Requirements	Scenarios					
			Individual/Small Team	Manned Ground Vehicle	Field/Contingency		Fixed Base	
			Test Raw Water	Test Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
	Collection Method	Continuous flow or grab sample?	Grab	Grab	Grab	Continuous	Grab	Continuous
13	Robustness	If reusable, do or can TICs accumulate on device from test to test (to create a false positive)?	No (T)	No (T)	No (T)	No (T)	No (T)	No (T)
14	Robustness	Device has a indicator that ESB system is still working (or has a malfunction signal)?	Yes (T)	Yes (T)	Yes (T)	Yes (T)	Yes (T)	Yes (T)
15	Robustness	Number of tests (or hours if using continuous collection method) before reloading, recalibration?	One test (T), 150 tests (O)	One test (T), 150 tests (O)	One test (T), 150 tests (O)	20 hrs (T), 168 hrs (O)	One test (T), 150 tests (O)	24 hrs (T), 168 hrs (O)
16	Robustness	Reliability? Device failure rate per X amount of tests (or time if using continuous collection method)?	one per 850 tests (T & O)	one per 850 tests (T & O)	one per 850 tests (T & O)	280 hrs (20 hrs/day for 14 days) (T) 560 hrs (20 hrs/day for 28 days) (O)	one per 850 tests (T & O)	30 days (T), 60 days (O)
17	Robustness	Shelf life (device and consumables)?	1 yr (T), 2 yr (O)	1 yr (T), 2 yr (O)	1 yr (T), 2 yr (O)	1 yr (T), 2 yr (O)	30 days (T), 1 yr (O)	30 days (T), 1 yr (O)
18	Safety, Health, & Environmental	What are the requirements related to the safety, health of the user and the environmental impact?	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)	No safety hazard to logistics or operational personnel (T). Complies with DoD HazMat Directives (T)

APPENDIX C

ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or maximum requirement (O1 is a higher priority than O2)

#	Category	Tech. Requirements	Scenarios					
			Individual/Small Team	Manned Ground Vehicle	Field/Contingency		Fixed Base	
			Test Raw Water	Test Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
	Collection Method	Continuous flow or grab sample?	Grab	Grab	Grab	Continuous	Grab	Continuous
19	Testing Characteristics	Min. false positive readings and false negative readings (for specified chemicals). *But which is more important* (how much)?	false + <1/1000 (T), <1/5000 (O)false - <1/5000 (T), <1/10,000 O)	false + <1/1000 (T), <1/5000 (O)false - <1/5000 (T), <1/10,000 O)	false + <1/1000 (T), <1/5000 (O)false - <1/5000 (T), <1/10,000 O)	false + <1/1000 (T), <1/5000 (O)false - <1/5000 (T), <1/10,000 O)	false + <1/1000 (T), <1/5000 (O)false - <1/5000 (T), <1/10,000 O)	false + <1/1000 (T), <1/5000 (O)false - <1/5000 (T), <1/10,000 O)
20	Testing Characteristics	Minimum time between consecutive tests (assumes reusable device)	30 min (T), Immediate (O)	Time to refill reservoir (T), Time to produce 2 liters of water (O)	1 hr (T), Immediate (O)	N/A	1 hr (T), Immediate (O)	N/A
21	Testing Characteristics	Sample prep complexity (e.g., measured volume, reagent addition)	None (T) None (O)	<5 steps (T), none (O)	raw: 2 steps (T) none(O) Treated: 5 steps (T), none (O)	None after initial set up	<5 steps (T), none (O)	None after initial set up
22	Testing Characteristics	What is the test turn around time (time from starting test [i.e., includes set-up time] until knowing the results) ?	1 min (T) 15 sec (O)	2 min (T) 15 sec (O)	10 min (T) 2 min (O)	5 min (T) 1 min (O)	10 min (T) 5 min (O)	10 min (T) 5 min (O)
23	Testing Characteristics	Time to complete test (operator time to set up and sample)?	30 sec (T) 5 sec (O)	1 min (T) 30 sec (O)	5 min (T) 30 sec (O)	None after initial set up	5 min (T) 2 min (O)	None after initial set up
24	Testing Characteristics	Type of test results needed: Go-No Go vs. performing a procedure (e.g., look up/analysis)?	Simple (Go-No Go) (T)	Simple (Go-No Go) (T)	More Complicated than Go-No Go (T), Go -No Go (O)	More Complicated than Go-No Go (T), Go -No Go (O)	More complicated than Field/ Contingency Threshold (T), Go-No Go (O)	More complicated than Field/ Contingency Threshold (T), Go-No Go (O)

APPENDIX C

ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNICAL REQUIREMENTS FOR USER SCENARIOS

Notes: "T" means threshold or minimum requirement; "O" means objective or maximum requirement (O1 is a higher priority than O2)

#	Category	Tech. Requirements	Scenarios					
			Individual/Small Team	Manned Ground Vehicle	Field/Contingency		Fixed Base	
			Test Raw Water	Test Treated Water	Test Raw/ Treated Water	Test Treated Water	Test Raw/Treated Water	
	Collection Method	Continuous flow or grab sample?	Grab	Grab	Grab	Continuous	Grab	Continuous
25	User Requirements	What is the level of skills and knowledge required to perform maintenance and calibration tests (field thus soldier/ simple or depot thus technician/ moderately complex)?	None (T) None (O)	None (T) None (O)	Medium (T) Low (O)	Medium (T) Low (O)	Moderate (T) Medium (O)	High (T) +Moderate (O)
26	User Requirements	What is the level of skills and knowledge required to perform tests (soldier/simple vs. technician/moderately complex)?	Low (soldier simple) Dip, read, interpret result (go/no-go) (T,O)	Low (soldier simple) Fill sample vial, add reagent, dip, read, interpret result (go/no-go)	Medium (T) Low (O)	Medium (T) Low (O)	Moderate (T) Medium (O)	High (T) +Moderate (O)

APPENDIX D

ADDITIONAL INFORMATION ON MILITARY EXPOSURE GUIDELINES

Military Exposure Guidelines (MEGs) are provided for water (and other media) to estimate a level "... above which certain types of health effects may begin to occur in individuals amongst the exposed population". MEGs are "... designed to indicate 'thresholds' for minimal to no adverse health effects" and are considered "... protective against any significant non-cancer effects". MEG exposure scenarios for water are appropriate for a deployed military operation, i.e., exposure lasting either 5 or 14 days, with water consumption of either 5 or 15 L/day. The exposed population is defined to include "... relatively healthy and fit male and non-pregnant female adults", 18 to 55 years old with and average weight of 70 kg. It is important to note that while MEGs are not enforceable military standards, the MEGs are considered guideline concentrations for identifying and ranking Occupational and Environmental Health (OEH) risks. MEGs have been established for about 190 chemicals. MEGs provide a reference point above which adverse effects may be expected after a field-relevant period of exposure and may serve as lower thresholds for toxicity sensor responses. That is, responses at concentrations below the MEGs indicate toxic effects that may not be relevant to acute human health impairments.

Reference: U.S. Army Center for Health Promotion and Preventive Medicine. 2001. Chemical Exposure Guidelines for Deployed Military Personnel. TG-230. U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD.

Blank

APPENDIX E

POSSIBLE ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNOLOGIES			
#	Technology	Technology System	Technology Category
1	AquaSentinel	Algae	Algae
2	Fluotox	Algae (Scendedesmus subspicatus)	Algae
3	Lumitox	Algae (Pyrocystis lunula)	Algae
4	Amtox	Bacteria	Bacteria
5	Baroxymeter	Bacteria	Bacteria
6	BioTox Flash Test	Bacteria (Vibrio fischeri)	Bacteria
7	Cellsense	Bacteria, algae	Bacteria
8	GreenScreen EM	Yeast (Saccharomyces cerevisiae)	Bacteria
9	LUMIStox	Bacteria (Vibrio fischeri)	Bacteria
10	MetPlate	Bacteria (Escherichia coli)	Bacteria
11	Microtox, Deltatox	Bacteria (Vibrio fischeri)	Bacteria
12	Polytox	Bacteria	Bacteria
13	*Sinorhizobium meliloti* Toxicity Test	Bacteria (Sinorhizobium meliloti)	Bacteria
14	STIPTOX	Bacteria	Bacteria
15	Stressor-Specific E. coli Sensor	Bacteria (Sinorhizobium meliloti)	Bacteria
16	Toxi-Chromo Test	Bacteria (Escherichia coli; heavy metal sensitive variant)	Bacteria
17	ToxScreen	Bacteria (Photobacterium leiognathi)	Bacteria
18	ToxTrak	Bacteria	Bacteria
19	Vitotox	Bacteria (Salmonella tyhimurium)	Bacteria
20	Aquanox	Enzyme (horseradish peroxidase)	Enzyme
21	Eclox	Enzyme (horseradish peroxidase)	Enzyme
22	Mitoscan	Enzyme (sub-mitochondrial particles)	Enzyme
23	ArrayScan HCS System	Mammalian cells	Mammalian cells
24	Brain on a Chip	Mammalian cells	Mammalian cells
25	CANARY Biosensor	Mammalian cells (B Lymphphocytes)	Mammalian cells
26	Cell Assay	Mammalian cells	Mammalian cells
27	Cell Culture Analog	Mammalian cells (lung, liver, others)	Mammalian cells
28	Cytosensor Microphysiometer	Mammalian cells	Mammalian cells
29	Epithelial Cell Toxicity Sensor	Mammalian cells (epithelial)	Mammalian cells

30	Multianalyte Micro- and Nano- Physiometers	Mammalian cells	Mammalian cells
31	Neurochip Biosensor System	Mammalian cells (neurons)	Mammalian cells
32	Portable Cell-Based Biosensor	Mammalian cells (cardiomyocytes)	Mammalian cells
33	Portable Neuronal Microelectrode Array	Mammalian cells (neurons)	Mammalian cells
34	SOS Cytosensor System	Fish cells (chromato-phores)	Mammalian cells
35	ToxiLight	Mammalian cells	Mammalian cells
36	*C. elegans* Monitor	Multicelleular: *Nematode (Caenorhabditis elegans)*	Multicelleular
37	Instant Multicellular Organisms	Multicelleular (Fish aquatic invertebrates, and algae with a life cycle resting stage)	Multicelleular
38	IQ Toxicity Test	Multicellular: Daphnid (Daphnia magna)	Multicelleular

APPENDIX F

EXAMPLE OF AN ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNOLOGY FACT SHEET

Microtox, Deltatox

(1) Technology description

a. Vendor
Strategic Diagnostics Inc.
111 Pencader Drive, Newark DE 19702-3322 USA
Sales: (800) 544-8881 Tel: (302) 456-6789 Fax: (302) 456-6782
(http://www.sdix.com/ProductSpecs.asp?nProductID=7)

b. Toxicity sensor type

i. Living system used: *Vibrio fischeri* (luminescent bacteria)

ii. Endpoint monitored: Inhibition of light output or toxicity threshold; light is produced as a byproduct of cellular respiration.

iii. Monitoring method

1. Freeze-dried bacteria are reconstituted in salt solution containing water sample to be tested
2. Luminescence readings are taken prior to and at 5 and 15 min. after water sample addition

iv. Continuous monitoring or grab sample? Grab sample

v. Note: Microtox and Deltatox generally produce similar results; Deltatox is designed to be field portable and lacks the temperature control capabilities of Microtox.

(2) Logistical considerations

a. Environmental requirements: Testing done in salt water solution; temperature is maintained at 15 deg. C; ambient temperatures must be 15-30 deg. C.

b. Storage and transport: Shelf life of freeze-dried bacteria 1 year when stored at -20 to -25° C; less at normal refrigerator temperatures.

c. Sample throughput: Microtox: 15 samples per hour; Deltatox: 20 per hour

d. Cube and weight: Microtox: 1.0 ft^3; 21 lbs; Deltatox: 0.2 ft^3, 6 lbs

(3) Response to toxic chemicals (EPA ETV report available)

a. Chemicals tested: extensive database; see http://www.sdix.com/pdf/Toxicity%20Data%20Index.pdf; also tested as part of MEIC in vitro cytotoxicity evaluation program; see graphs on next page

b. Toxicity data:

i. ETV results: no inhibition up to human lethal levels for botulinum toxin, ricin, soman, and VX; aldicarb, colchicine, cyanide, dicrotophos, and thallium were detected at or below lethal levels

ii. Response relative to human lethal values and MEGs: See graph on next page

c. Test precision: Range of test standard deviations from ETV evaluation were most less than 10%.

d. Interfering substances and water conditions:

i. Samples must be dechlorinated before testing; chloraminated water may interfere after dechlorination
ii. Turbid and colored samples may require pre-treatment; pH outside range 6.0 – 8.0 requires adjustment
iii. ETV testing showed copper and zinc to cause interferences

Literature Cited

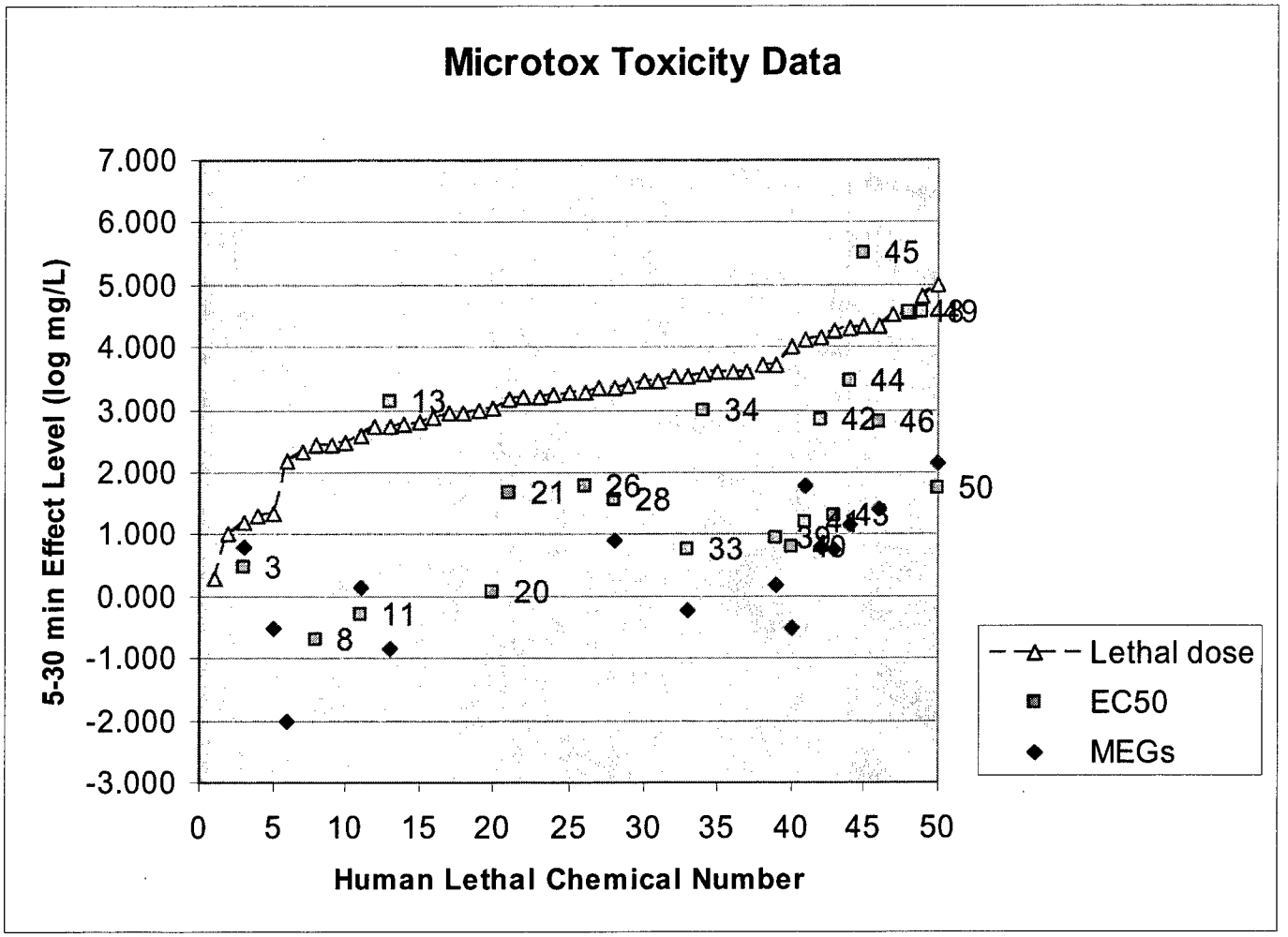
Clemedson C, McFarlane-Abdulla E, Andersson M, Barile FA, et al. 1996. MEIC evaluation of acute systemic toxicity Part I. Methodology of 68 *in vitro* toxicity assays used to test the first 30 reference chemicals. *ATLA* 24:251-272.

Clemedson C, Andersson M, Aoki Y, Barile FA, et al. 1998. MEIC evaluation of acute systemic toxicity Part IV. *In vitro* results from 67 toxicity assays used to test reference chemicals 31-50 and a comparative cytotoxicity analysis. *ATLA* 26:131-183.

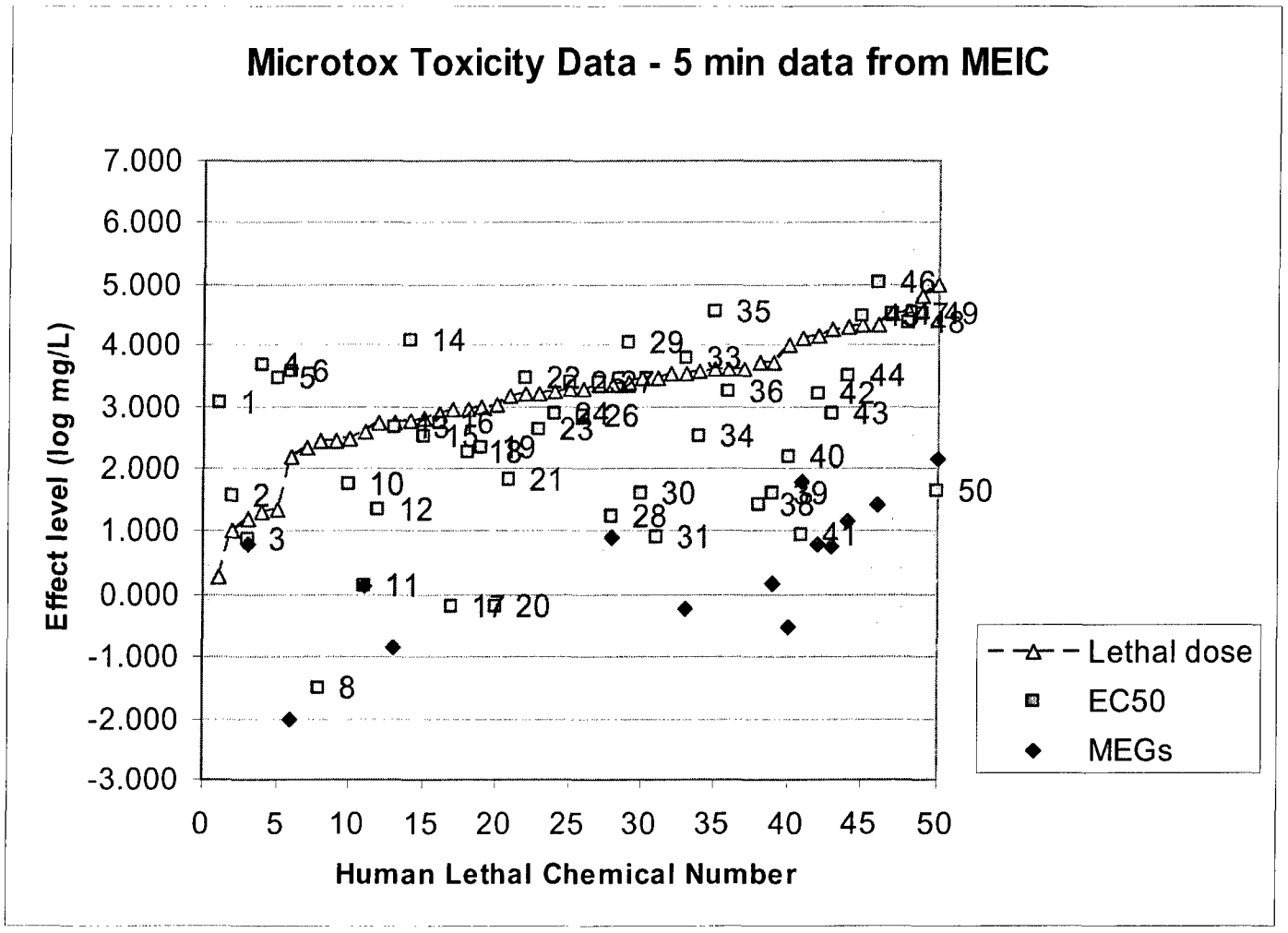
Kaiser KLE, Palabrica VS. 1991. *Photobacterium phosphoreum* toxicity data base. *Water Pollution Research Journal Canada* 26:361-431.

U.S.Army Center for Health Promotion and Preventive Medicine. 2001. Chemical Exposure Guidelines for Deployed Military Personnel. TG-230. U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD.

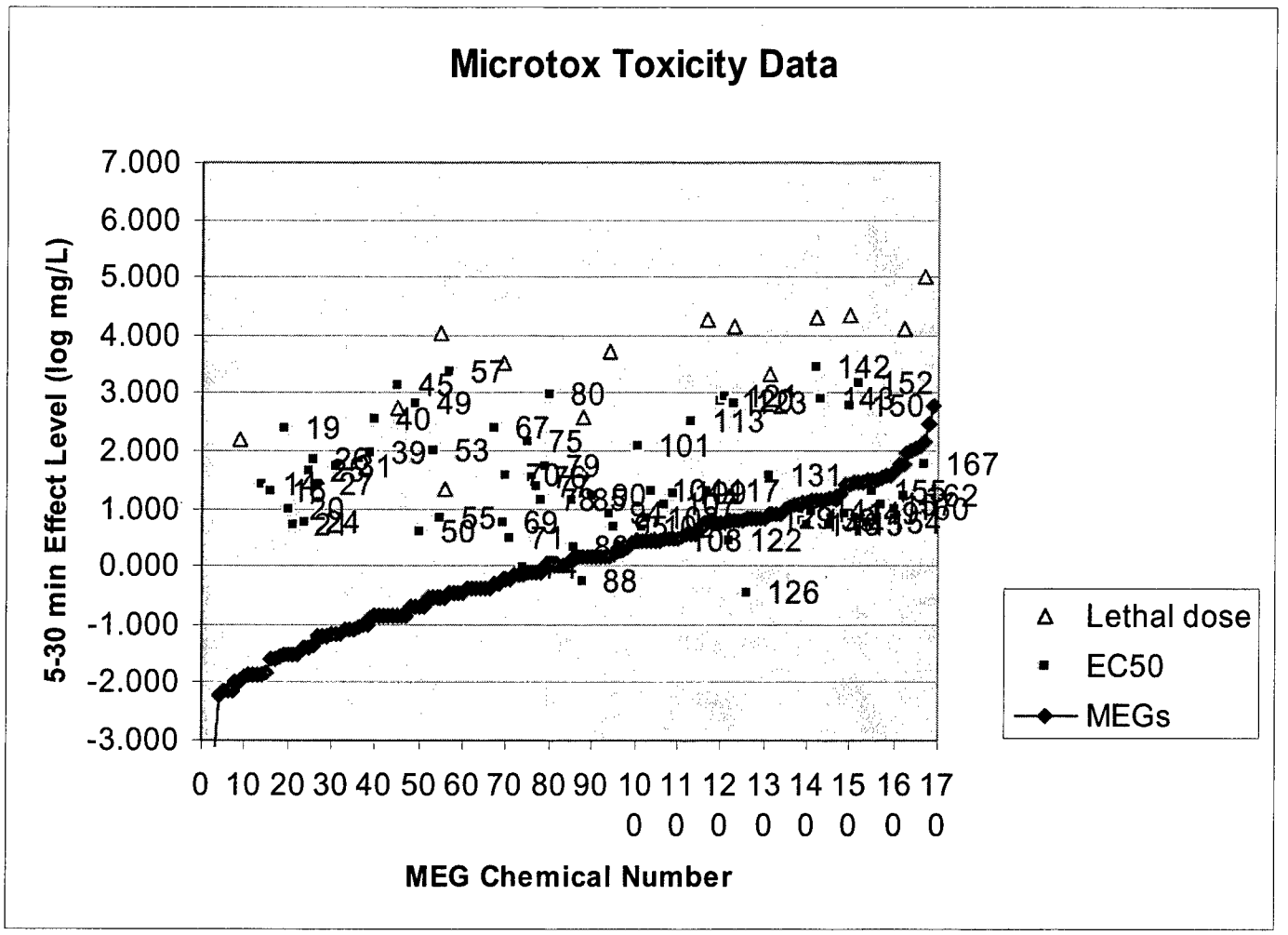
Walum E. 1998. Acute oral toxicity. *Environmental Health Perspectives* 106:497-503.
Ref Type: Journal



Lethal doses for 50 chemicals (Wallum, 1998) are plotted in order of increasing toxicity as concentrations in water, assuming a 70 kg person consuming 5 L of water. Military Exposure Guidelines (MEGs) for water (5 day, 5 L/day) are available for 15 of the 50 chemicals (USACHPPM, 2001). Microtox data from Kaiser and Palabrica, 1991.



Lethal doses for 50 chemicals (Wallum, 1998) are plotted in order of increasing toxicity as concentrations in water, assuming a 70 kg person consuming 5 L of water. Military Exposure Guidelines (MEGs) for water (5 day, 5 L/day) are available for 15 of the 50 chemicals (USACHPPM, 2001). Microtox data from Clemedson et al., 1996, 1998.



Military exposure guidelines (MEGs) for water (5 day exposure, 5 L/day consumption) for 169 chemicals (USACHPPM, 2001) are plotted in order of increasing toxicity. Corresponding human lethal concentrations derived from lethal dose levels (Wallum, 1998) are available for 15 of the 169 chemicals and assume a 70 kg person, 5 L consumption. Microtox data from Kaiser and Palabrica, 1991.

Blank

APPENDIX G

ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM DOWNSELECT STRAWMAN MODEL GOALS AND MEASURES

Note: This is a working document (4-22-04 version) and is not complete. The model goals, measures, scales, and criteria weights will be finalized after all needed data from the additional tests become available. We do this because the testing result differences between each ESB system are critical to how the model is set-up (e.g., if there's no difference between the ESB technologies for one criterion, using this criterion does not help in the decision making process).

The numbers within the parenthesis refer to the numbers in the ESB system technical requirements for user scenario document (e.g., 4 is "what's the level of detection for each chemical", this is a performance criteria; see Appendix 3).

1. **Performance** – how well does the technology work?
   a. Chemicals Detected:

      i. (3, 4) The ability of the technology to provide an adverse response to a set of representative chemicals at concentrations closest to the short-term MEG for the greatest number of test chemicals, without being above the lethal level or appreciably below the MEG. *It is better to respond to the greatest number of chemicals closest to each short-term MEG without responding appreciably below the MEG or above the lethal level.* Need to weight certain chemicals more based on modes of action, what is important to users [e.g., CNS]. Needs to be finalized. A false negative is more suitable to the user's need than a false positive (individual soldier scenario).

      Same for all 4 scenarios
      Scale:
      100 - x Chemicals
      0 - x Chemical

      The list of chemicals will reflect the prioritization set by the users. We have to keep in mind what the filtration system will remove from the water per scenario.

   b. Water Characteristics:

      i. (6, 7) The ability to operate under a number of water quality conditions and in the presence of interfering substances (e.g., quantity of total dissolved solids, turbidity, chlorine residual, ammonia, and metals [e.g., copper], pH, temperature) with minimal effect on test outcome. *It is better to be able to operate under a wide range of water quality conditions than under a more restricted range.*

      Same for all 4 scenarios, assumes reasonable sample preparation for all scenarios except for individual soldier scenario (they will do no preparation)

Better method: (scoring should reflect ability plus ease of correctability [must carry through requirements to other measures within model])

Considering these factors – cations, copper, ammonia, chlorine, chloramines, coagulants, dissolved solids, turbidity/clay, pH, temp, the experts will use this scale:

100 –High, means able to operate under a wide range of conditions
0 – Low, means able to operate under a very restricted range of conditions

c. Reliability

i. Technology Failure Rate (17) The number of times a technology fails to produce a result per a certain number of tests. By failure we mean the technology, not a human mistake. *It is better to have fewer rather many failures.*

May be a measure for an ESB system, not for individual technologies. All scenarios.

Scale:
100- means a low failure rate
50 - Medium
0- means a high failure rate

ii. Test Reproducibility (20) Need definition. *It is best to have high reproducibility.*

For all scenarios
Scale:
100-High
50-Medium
0-Low

iii. Operational Indicator (15) Technology includes an indicator to show that it is operational. *It is better if the technology system has an automatic malfunction signal rather than a manual or more elaborate method to detect a malfunction or a system not working properly.* Assume all technologies have an operational indicator?

For all scenarios
Scale:
100-has operational indicator, easy to use (automatic)
50- has operational indicator, not easy to use
0- does not have an indicator

iv. Positive Control Indicator, Positive control is essential in fixed base scenario. Need to develop definition.

For all scenarios
Scale:

100-has positive control indicator, easy to use (automatic)
50- has positive control indicator, not easy to use
0- does not have an indicator

2. **Operational Impact** – how does the technology impact other operations?

a. Test Turn Around Time

i. (23, 24) The time required between consecutive tests (assumes reusable technology). Includes operator set-up time, sample preparation, sensor operation time, and any time required for the system to reset before another reading. *It is better to have less rather than longer time required to perform tests.* If it takes more than 30 minutes this would earn a very low score for the IS scenario.

IS Scenario
Scale:

100- 20 Seconds (or best technology)
0- 1.5 Minutes (or worst technology)

FB-FC-MGV Scenario
Scale:

100- 7 Minutes
0 - 15 Minutes

b. Environmental Conditions

i. (5) The range of environmental conditions under which the technology can properly operate. These conditions include air temperature, and the humidity level's operable range for the technology. *It is better to be able to operate under all environmental conditions and extremes.*

FB-FC-MGV Scenario
Scale:

100- High, means able to operate under a wide range of conditions (User will refine temperature and humidity requirements)
0- Low, means able to operate under a very restricted range of conditions

IS Scenario
Scale:

100- High, means able to operate under a wide range of conditions (5-95% RH & 0-45C)
0- Low, means able to operate under a very restricted range of conditions (

Better method: (scoring should reflect ability plus ease of correctability [must carry through requirements to other measures within model])

Considering these factors – ambient operational temperature (most important factor), relative humidity

c. Ease of Use

i. Sample Preparation and Skills Required (22, 26, 27) The sample preparation and performing test complexity level (e.g., measured volume, reagent addition). The focus is not on how long it takes to perform each task but rather how complicated each task is. *It is better for the tasks to be simple (a soldier can perform) rather than complex (a technician must perform). It is better for the tasks to be simple (a soldier can perform) rather than complex (a technician must perform at a depot).*

FB-FC Scenario
Scale:

100- few steps of preparation, minimal skill level
80 (place holder)- some steps of preparation, moderate skill level (NCO and above, water technician with lab skills and lab capabilities)
0- many steps of preparation, significant skill level (senior water technician with lab skills and lab capabilities)

IS-MGV Scenario
Scale:

100- no sample prep, minimal skill level (does not have to think about it, can be trained [15 minutes of training or less]
0- some sample prep, a little more than minimal skill level (does not have to think about it, can be trained [15 minutes of training or less]

ii. Ease of Data Interpretation (25) The ease of making the data interpretable (go/no-go vs. look up/analysis). *It is better to have test results that are not ambiguous.*

FB-FC Scenario
Scale:

100- Go/No-go, test results that are not ambiguous
50 -
0- Moderate look up/analysis (lab technician skills and experience would be required, soldier must go to a reference to look up result)

IS-MGV Scenario
Scale:

100- Go/No-go, test results that are not ambiguous

50 -
0- Minimal look up/analysis (interpret a numerical reading)

3. **Logistics**
   a. Size and Weight

      i. (9, 12) The cube, maximum length, and weight of the technology and necessary peripheral equipment (e.g., maintenance and repair supplies and parts, power source, and a 2-week supply of consumables). *It is better for the overall weight and cube to be less rather than more. Max. - perform 8 tests per day per person (individual soldier scenario).*

FB-FC-MGV Scenario
Scale:
100-less than or equal to 1.5 cu ft, less than or equal to 12.5 lb
50- 1.5-3 cu ft, 12.5-25 lb
25 -....
0- more than 6 cu ft, more than 40 lb

IS Scenario
Scale:
100-8 oz or less, 3"x3"x2"
0-greater than a 16 oz, bigger than 6"x8"x10"

   b. Ruggedness (May want to delete this GOAL and just have Shelf Life as "c")

      i. Shelf Life (18) The storage life of the technology and consumables (i.e., how long will they be usable [includes shipping time] and under what conditions must they be stored). *It is better when the technology and its consumables have a long shelf life and there are minimum storage requirement/restrictions.*

FB Scenarios
Scale:
100- more than 6 months, under these conditions (without a environmental control required)
80- more than 6 months, under these conditions (with environmental control required)
40-between 30-days and 6 months, under these conditions (with environmental control required)
0 - less than 5-day, under these conditions (with significant environmental control required)

FC-MGV-IS Scenarios
Scale:
100- more than 6 months, under these conditions (without a environmental control required)
0 - less than 30 day (includes 2-weeks shipping), under these conditions (must perform without environmental controls)

c. System Maintenance

i. Time complexity and materiel required to maintain test system. *Want minimum requirements for organism culture.*

FB Scenario
Scale:

100- requires no biological culturing
0- requires continuous biological culturing

FC Scenario
Scale:

100- requires no biological culturing
0- limited maintenance of culture (e.g., media replacement)

MGV-IS Scenarios (this is a screening tool)
Scale:

100- requires no biological culturing
0 - requires any biological culturing

4. **Programmatics** (Recommend evaluate subsequent to model application)

a. Maturity/Risk

i. How mature is this technology (e.g., Technology Readiness Level [need to send TRLs to everyone])? Are there a lot of data gaps for this technology? Can the data be obtained, or does a lot of testing still have to be done to acquire it? How likely would a high success rate be for this technology?

Scale:
100-
0-

b. Cost

i. How costly would this technology be to fund/develop? Are there a lot of costs involved for the testing process (cost per test, not to include consumables)? Are there a lot of costs involved with supporting/peripheral equipment/protective gear? Development cost (filling in data gaps, etc), reoccurring annual costs (cost per test (variable costs), storage costs (fixed/sunk costs), etc), and life-cycle.

Scale:
100-
0-

User technical requirements not within model:

Screening threshold questions: 1 (grab vs. continuous) - scenario dependent, recommend focusing on (grab) requirement only

Taken out of model (not to be used for any screening):

21 - taken out of model because group does not believe it is critical/a discriminator.

10,11 - Ease of Controls/Display (10, 11) How easy it is to prepare and perform the tests to include using the technology's controls (e.g., dials, switch, knob) and reading the display (be able to read the results) in low light conditions while wearing and working in protective and cold weather suits. *The easier and less time consuming to perform these tasks the better.* **This is an engineering issue?** – taken out of model because group does not believe it is critical/a discriminator

16 - Consecutive Tests Performed (16) The number of tests that can be completed before reloading or recalibration of the technology (assumes reusable technology). *It is better to have many rather than fewer tests that can be performed before reloading or any maintenance function.* taken out of model because group does not believe it is critical/a discriminator

13 - Water/Impact Resistance (13) The ability of the technology to produce usable results after it is immersed in water for 30 minutes or is dropped onto concrete from a height of 3 feet **(can we limit this consideration to just the individual soldier/small team and MGV scenarios?).** *It is better for the technology to function after immersion in water or after being dropped.* **Is this more of an engineering issue?** taken out of model because group does not believe it is critical/a discriminator.

2 - (fixed vs. sliding scale) – recommend focusing on threshold (fixed level) requirement only.

19 - (safety, health, and environment) – not likely to be useful as a discriminator between technologies.

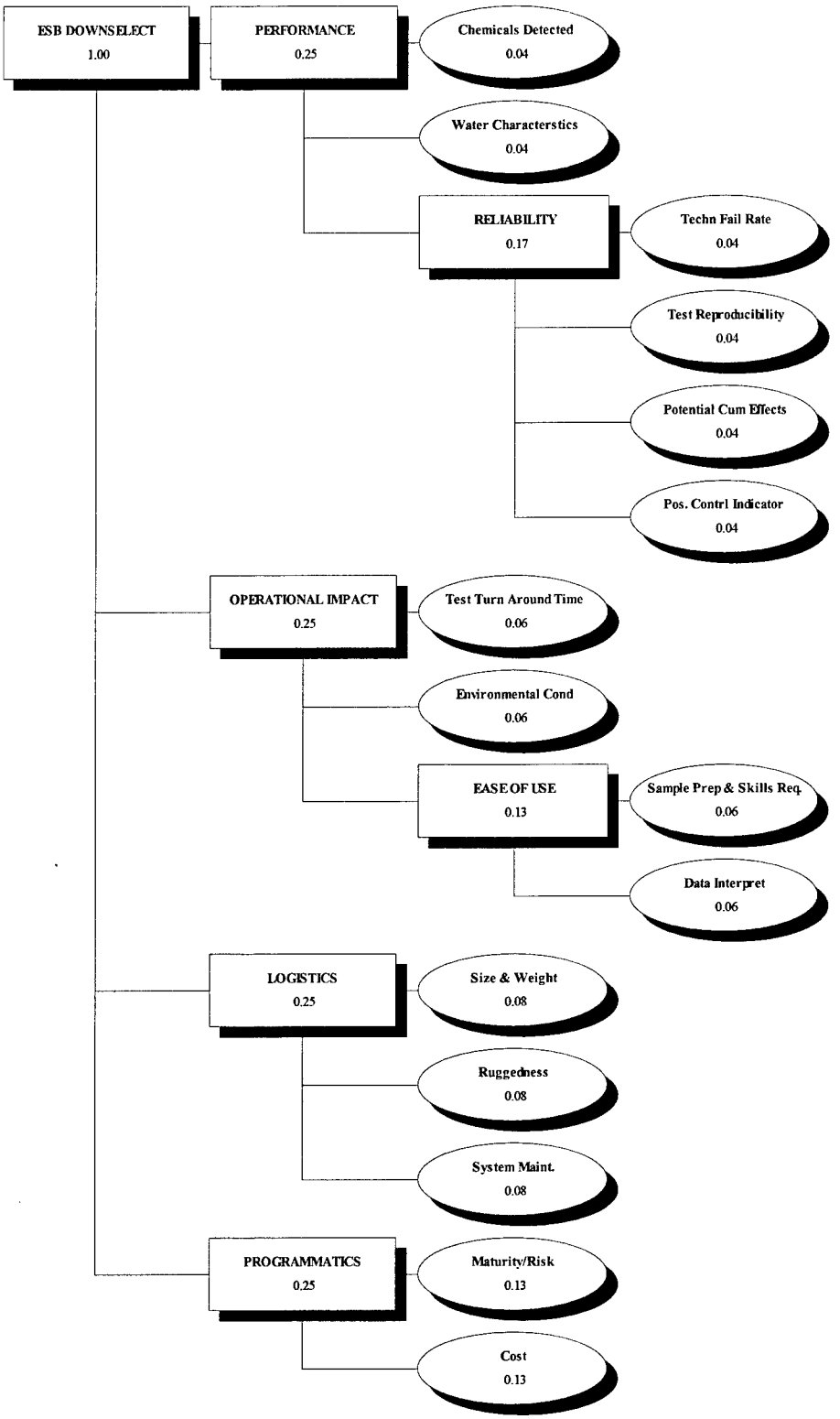
14 - Potential for Carryover Effects (14) The ability of the technology to not allow cumulative exposure to TICs to produce a false positive result (assumes reusable or continuous technology). *It is better when cumulative exposures to TICs do not produce a false positive result.* Another concern is the "masking" effect. The technology has been exposed to a masking TIC and has been "effected" so it does not detect like it did prior to being exposed to the masking TIC. Cannot get needed information within next 6-12 months.

8 - Power Requirements (8) The amount and source of electrical power (e.g., ampere, watts) required for the technology for two weeks of operation. *It is better to have less power required and the ability to use battery along with other power sources. May only be a measure for individual soldier scenario and field operations. Logisitcs of power source is covered in size and weight measure.* Focus of measure should only be on how power is

required and not the source of the power (renewable, replaceable). It's best for the technology to not require any power.

ESB System Strawman Model (next page)

Notes: Weights are only given as examples. User representatives have not addressed weights for goals or measures yet. The user representatives will finalize the model after test results for the 9 ESB system technologies being considered become available in FY05.


ESB DOWNSELECT
1.00
PERFORMANCE
0.25
Chemicals Detected
0.04
Water Characterstics
0.04
RELIABILITY
0.17
Techn Fail Rate
0.04
Test Reproducibility
0.04
Potential Cum Effects
0.04
Pos. Contrl Indicator
0.04
OPERATIONAL IMPACT
0.25
Test Turn Around Time
0.06
Environmental Cond
0.06
EASE OF USE
0.13
Sample Prep & Skills Req.
0.06
Data Interpret
0.06
LOGISTICS
0.25
Size & Weight
0.08
Ruggedness
0.08
System Maint.
0.08
PROGRAMMATICS
0.25
Maturity/Risk
0.13
Cost
0.13

Blank

APPENDIX H

EVALUATION METHOD FOR ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM HIGH-LEVEL QUALITATIVE MODEL

Evaluation Method: A two-step process will be used. The first step is to reduce the number of technologies for further consideration and then to rate and then rank the remaining technologies (or top 20).

Step 1: Rate each ESB technology using the rating categories below.

Rating Categories:

Green – means the technology is promising. Final rating and ranking will be completed in step 2.

Orange – means the technology may be promising at some point but (and) the technology is currently immature and needs more R&D (e.g., the technology is not to the point in development where testing can be completed to determine toxicity sensitivity). The technology will not be considered in step 2 but is put on a technical watch list.

Red – means the technology is not promising or not reasonably projected to ever be promising. A technology earns a red rating for any of the reasons below and is eliminated from further consideration.

- Technology toxicity sensitivity is inappropriate or the technology does not provide a unique end-point (i.e., metabolic, physical response)

- Technology is redundant and inferior to another technology.

- Technology is unfeasible/not viable. It does not or is not expected to meet a minimum requirement of a user scenario.

  -- Technology takes too much time (hours) to produce an end-point.

  -- Technology requires pre-culturing (e.g., requires actively taking out and culturing organism for a long period of time)

Step 2: Evaluate all technologies that were rated green in step 1. Rate each ESB technology against the criteria and rating categories below. After each technology is rated then rank the technologies (1-n).

Rating Categories:

Green – means the ESB technology is known to or is expected to perform "well" against this criterion's requirements or is "likely" to achieve this criterion's requirements.

Yellow – means the ESB technology is known to or is expected to perform only "ok" against this criterion's requirements or "may" achieve this criterion's requirements.

Red – means the ESB technology is known to or is expected to perform "poorly" against this criterion's requirements or is "not likely" to achieve this criterion's requirements.

Evaluation Criteria (see note 1):

- Technology provides an appropriate toxicity sensitivity response (SR) and/or provides unique information (e.g., unique end-point).

- Technology is only minimally affected by interferences (I). Interferences are turbidity, color, and ionic composition of the media.

- Technology is reliable/repeatable (R&R).

- Technology has an appropriate rapidity of response (RoR). A green rating is 20 min or less, yellow is 20-60 min, and red is over 60 min.

Note 1: Ranking decisions are based on the ability of the technologies to meet at least one of the user scenarios. The focus is primarily on appropriate toxicity response – at the correct level of sensitivity, in a reasonable period of time, and with the reproducibility and absence of interferences necessary to reach an appropriate evaluation of a test sample.

APPENDIX I
HIGH-LEVEL QUALITATIVE MODEL AND ASSESSMENT/RESULTS FOR ENVIRONMENTAL SENTINEL BIOMONITOR (ESB) SYSTEM TECHNOLOGIES

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
1a	20	Aquanox	Enzyme (horse-radish peroxidase)	Enzyme	Green-Go to Step 2	Appears to be redundant to Eclox - expert panel is unsure whether there are any advantages that would warrant it's inclusion in the program. Need to verify which of these two systems is preferred.	Green	Green	Green	Green	SR- too sensitive for some chemicals; I - very sensitive to cations and conductivity; R&R- CV less than 10% ; RoR- 5 minutes.
1b	21	Eclox	Enzyme (horse-radish peroxidase)	Enzyme	Green-Go to Step 2	Developed for military, ready to go now (see comments for Aquanox)	Green	Green	Green	Green	See comments for Aquanox.
2	11	Microtox, Deltatox	Bacteria (Vibrio fischeri)	Bacteria	Green-Go to Step 2	Commercial development and acceptance, widely used, history, lots of data available, field deployable unit available.	Green	Green	Green	Green	SR- more data is available for this than any other assay, with demonstrated sensitivity, but it missed some targets in ETV testing; I - minimally affected by interferences; R&R- false positives will be an issue; RoR- 5-15 min.

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
3	22	Mitoscan	Enzyme (sub-mito-chondrial particles)	Enzyme	Green-Go to Step 2	Mammalian system may provide some unique sensitivities.	Green	Yellow	Green	Green	SR- one of the best technologies under review; I - expected to be similar to Aquanox, but no data available; R&R- thought to be high, SMPs; RoR- range of 5-20 minutes
4	17	ToxScreen	Bacteria (Photo-bacterium leiognathi)	Bacteria	Green-Go to Step 2	Good complement to Microtox (different bacterium with possible sensitivity differences)?	Green	Green	Green	Yellow	SR- more sensitive than Microtox in ETV testing; two buffer system required (one for metals and one for organics); I - about the same as Microtox ; R&R- within expected range for other similar tests based on ETV testing; RoR- 30-60 minutes.
5	13	*Sinorhizobium meliloti* Toxicity Test	Bacteria (Sinorhizo-bium meliloti)	Bacteria	Green-Go to Step 2	Similar sensitivity as Microtox, but different toxicity endpoint	Green	Green	Green	Green	SR- most chemicals were detected within the target range; I - divalent cations is the main issue that has to be addressed (this is treatable); R&R- less than 25% CV in many cases; RoR- 20 min. or less.

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
6	34	SOS Cytosensor System	Fish cells (chromato-phores)	Mammalian cells	Green-Go to Step 2	Requires calibrant; lacks useful data (even though many chemicals have been tested).	Yellow	Yellow	Green	Yellow	SR- Not much data available. Did well at DARPA monitoring workshop for a variety of toxic chemicals; classification scheme available; I - An epithelial cell system that should be less susceptible to matrix-related interferences than mammalian cell systems ; R&R- estimated at +/- 30% ; RoR- Data reported from 1-100 minutes exposure; 60 min should not be a problem.
7	33	Portable Neuronal Micro-electrode Array	Mammalian cells (neurons)	Mammalian cells	Green-Go to Step 2	Concern with system's high sensitivity to electrolytes (calcium, magnesium) and endotoxin.	Yellow	Red	Green	Yellow	See comments for Portable Cell-based Sensor technology (Tech #32). Need to consider significance of endpoints (cardiac vs. neuronal).
8	32	Portable Cell-Based Biosensor	Mammalian cells (cardiomyo-cytes)	Mammalian cells	Green-Go to Step 2	Concern with system's high sensitivity to electrolytes (calcium, magnesium) and endotoxin.	Yellow	Red	Green	Yellow	SR- many unknowns, what is known is not compelling (but may offer unique detection for certain chemicals and endpoints not found in non-mammalian systems); I - high sensitivity to electrolytes (calcium, magnesium) and endotoxin ; R&R- expected to be good; c.v.s of 20%; RoR- 30 min.

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
9	7	Cellsense	Bacteria, algae	Bacteria	Green-Go to Step 2	Cannot be used with substances that are electrochemically active (e.g., the organophosphorus pesticide chlorfenvinphos); would need to check for the presence of this type of chemical by using electrodes without the associated immobilized bacteria.	Green	Green	Yellow	Yellow	SR- data is limited, what is available falls within the MEG/lethal concentration range; I - turbidity and color ok, but there may be issues with electrochemical activity; R&R- screen printed electrodes reduce reproducibility; RoR- 30 min or less
10	4	Amtox	Bacteria	Bacteria	Green-Go to Step 2	Must add ammonia to influent line; immobilized bacteria; not portable, but within minimum size requirements; capability for continuous monitoring.	Yellow	Yellow	Yellow	Green	SR- sensitivity will be variable; I - no information found; R&R- no information found; RoR- concentration dependent, but may be on the order of a 5-20 minute response cycle.

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
11	16	Toxi-Chromo Test	Bacteria (Escherichia coli; heavy metal sensitive variant)	Bacteria	Green-Go to Step 2	If mutant, it's rated Red. Unique pathway (gene expression)?	Yellow	Yellow	Yellow	Red	SR- Reported to be less sensitive than Microtox; I - no info available, but colormetric so turbidity will be a problem; R&R- no info available, but probably reproducible (E coli) but thought to be similar to Microtox; RoR- 90 minutes.
12	2	Fluotox	Algae (Scendedesmus sub-spicatus)	Algae	Green-Go to Step 2	Fluotox is the best of the algal based systems, none of which is particularly compelling.	Red	Yellow	Yellow	Yellow	SR- probably most sensitive to herbicides, but may have limited sensitivity to everything else; I - Optical technique, so turbidity is a concern; R&R- relying on vendor data; RoR- continuous monitor, so concentration dependent.
13	23	ArrayScan HCS System	Mammalian cells	Mammalian cells	Green-Go to Step 2	This will not be a standalone technology for the ESB mission - it is a benchmarking tool. There may be a database associated with this system that would be useful for identification of cellular targets. Need more info.	Yellow	Yellow	Green	Red	SR- lacking information, and will require sophisticated lab resources (not field deployable); I - lacking information; R&R- will be within 15-20% range; RoR- over 60 minutes.

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
See Note 1	29	Epithelial Cell Toxicity Sensor	Mammalian cells (epithelial)	Mammalian cells	Green-Go to Step 2	SBIR program will provide data that won't cost the ESB STO.	Red	Yellow	Yellow	Yellow	SR- very little data (limited to surfactants that attack the cell wall), no good comparison to other technologies, expected to be poor with 30 minute exposure (cell does not have time to manifest response); I - expected to be similar to other mammalian cell systems; R&R- similar to other mammalian cell systems; RoR- 30 minutes.
	15	Stressor-Specific E. coli Sensor	Bacteria (Sinorhizobium meliloti)	Bacteria	Orange - Tech Watch	Potential specificities for different modes of toxic action. Multiple engineered strains.					
	27	Cell Culture Analog	Mammalian cells (lung, liver, others)	Mammalian cells	Orange - Tech Watch	This project would be a good marker to watch other developments in this field.					
	30	Multianalyte Micro- and Nano-Physio-meters	Mammalian cells	Mammalian cells	Orange - Tech Watch	Lots of potential, but deep in R&D and will not be available for several years.					

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
	36	*C. elegans* Monitor	Multicelleular: *Nematode (Caenorhabditis elegans)*	Multicellular	Orange - Tech Watch	No evidence that system will ever be sensitive enough. Others (NIEHS) are investigating C. elegans as an environmental monitoring system.					
	37	Instant Multicellular Organisms	Multicelleular (Fish aquatic invertebrates, and algae with a life cycle resting stage)	Multicellular	Orange - Tech Watch	Potential issues - absorption, identifying endpoint, and enzyme activity.					
	1	Aqua-Sentinel	Algae	Algae	Red - Eliminated	Reliability/availability of test organisms is a concern.					
	3	Lumitox	Algae (Pyrocystis lunula)	Algae	Red - Eliminated	Reliability/availability of test organisms is a concern					

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
	5	Baroxy-meter	Bacteria	Bacteria		Skeptical about respiration as an endpoint. Concern with matrix effects - it may be necessary to match nutrient (organic) levels between the unknown sample and the reference sample to allow comparisons of respiration rate.					
	6	BioTox Flash Test	Bacteria (Vibrio fischeri)	Bacteria		Deltatox and Microtox would be more promising Vibrio fischeri based approaches, especially considering available sensitivity test results.					
	8	Green-Screen EM	Yeast (Saccharo-myces cerevisiae)	Bacteria							
	9	LUMIStox	Bacteria (Vibrio fischeri)	Bacteria		Deltatox and Microtox would be more promising Vibrio fischeri based approaches, especially considering available sensitivity test results.					

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
	10	MetPlate	Bacteria (Escherichia coli)	Bacteria		Limited scope of detection (just metals); requires 4-5 hr incubation period.					
	12	Polytox	Bacteria	Bacteria		Skeptical about respiration as an endpoint. Concern with matrix effects - it may be necessary to match nutrient (organic) levels between the unknown sample and the reference sample to allow comparisons of respiration rate.					
	14	STIPTOX	Bacteria	Bacteria		See comments for Baroxy-meter					
	18	ToxTrak	Bacteria	Bacteria		Best results are with cultures aged 10 to 72 hours.					

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
	19	Vitotox	Bacteria (Salmonella tyhimurium)	Bacteria		Extensive test time (3 hrs), prep time (overnight).					
	24	Brain on a Chip	Mammalian cells	Mammalian cells		Not practical - tissue can't be kept alive for longer than 12 hrs.					
	25	CANARY Biosensor	Mammalian cells (B Lymphpho-cytes)	Mammalian cells		Turn over technology to JSAWM project for further consideration					
	26	Cell Assay	Mammalian cells	Mammalian cells		Vendor non-responsive; sensor, no bio. Capturing cell in microfluidic system is impractical.					
	28	Cytosensor Microphysiometer	Mammalian cells	Mammalian cells		Not a stand-alone technology. End point is the measure of a calls viability.					
	31	Neurochip Biosensor System	Mammalian cells (neurons)	Mammalian cells		Technology gets folded into Portable Neuronal Microelectrode Array (Tech #33)					

Rank	#	Technology	Biological System	Technology Category	Step 1 Rating	Step 1 Rationale and Comments	Step 2 Rating				Step 2 Rationale and Comments
							SR	I	R&R	RoR	
	35	ToxiLight	Mammalian cells	Mammalian cells		Not a stand-alone technology. End point is the measure of a cells viability.					
	38	IQ Toxicity Test	Multi-cellular: Daphnid (Daphnia magna)	Multicellular		Too sensitive to be of practical use, takes too long, Daphnia is logisitically difficult.					

Note 1: Funded under a different program

Step 1 Ratings	Step 1 Reasons for Eliminating
Green-Go to Step 2	Inappropriate Sensitivity
Orange - Tech Watch	Redundant & Inferior
Red - Eliminated	Infeasible-time
	Infeasible-pre-culturing
	Infeasible-other
	Other
	Open

Step 2 Rating
Green
Yellow
Red

Blank

APPENDIX J

INFORMATION ON SELECTING TRAINING/TEST CHEMICALS AND LIST OF TRAINING/TEST CHEMICALS

Information on Selection of Training/Test Chemicals:

Considering the thousands of toxic industrial and agro-chemicals, an appropriate evaluation of toxicity sensor performance requires careful initial selection of the chemicals to be evaluated. Some possible criteria for selecting test chemicals include:

- Threat chemicals. Several organizations (U.S. Environmental Protection Agency (EPA), U.S. Army Research Development and Engineering Center (ARDEC)) and publications (Garland, 1991; Hickman, 1999; Clark and Deininger, 2000) have developed lists of threat chemicals for water supplies. Typically, the higher priority chemicals are highly toxic, soluble, persistent in water, and available in the region of concern. Chemicals high on these lists should be given priority as test chemicals for toxicity sensors.

- Mode of toxic action. Some chemicals can be grouped together because they cause similar effects on organisms. Examples include organophosphorus and carbamate insecticides (which cause acetylcholinesterase inhibition) and many neutral organic chemicals such as industrial solvents (which cause narcosis). To the extent that chemicals can be grouped in this way, it should be possible to select one or two representative chemicals from the group for testing to establish whether a toxicity sensor will respond to chemicals exhibiting the same general mode of toxic action.

- Chemical structural classes. Chemicals with certain structural, functional, or other similarities are frequently grouped together for testing purposes, such as heavy metals, algal toxins, or herbicides. Since chemicals within these groups may have distinctly different modes of toxic action, it is best to emphasize mode of toxic action categories in the selection of test chemicals. The MEIC chemical list provides a broad range of both modes of toxic action and chemical structural classes (Clemedson *et al.*, 1996, 1998).

- Interfering chemicals or water quality conditions. Interfering chemicals include substances commonly found in raw or treated water that may cause toxicity sensor responses at levels far below concentrations affecting humans, such as residual chlorine, copper, or ammonia. In addition, acceptable ranges of common water quality conditions, such as temperature, dissolved oxygen, conductivity, pH, turbidity, and microbial concentrations need to be defined for toxicity sensors to help minimize "false positive" responses.

Given the broad range of potential test substances, a compromise must be reached between minimizing the number of chemicals to conserve resources and expanding the number

to better define toxicity sensor response characteristics. One possible solution is the use of a tiered approach – conduct an initial screening of several toxicity sensor technologies with a few chemicals, followed by more thorough evaluation of technologies showing the most promise.

List of Training/Test Chemicals:

Chemicals are: Aldicarb *, Ammonia, Copper Sulfate, Mercuric Chloride *, Methamidophos *, Nicotine *, Paraquat Dichloride *, Pentachlorophenol *, Phenol, Sodium Arsenite *, Sodium Cyanide *, and Toluene.

Notes: "*" means it is a user-selected chemical

The responses of each toxicity sensor to residual chlorine and a high conductivity freshwater sample will be determined as well.

References

Clark RM, Deininger RA. 2000. Protecting the nation's critical infrastructure: The vulnerability of U.S. water supply systems. *Journal of Contingencies and Crisis Management* 8:73-80.

Garland JG. 1991. Water Vulnerability Assessments. AL-TR-1991-0049. Armstrong Laboratory, Occupational and Environmental Health Directorate, Brooks Air Force Base, TX.

Hickman, DC. 1999. A Chemical and Biological Warfare Threat: USAF Water Systems at Risk. Counterproliferation Paper No. 3. USAF Counterproliferation Center, Air War College, Maxwell Air Force Base, AL.

APPENDIX K

TEST PLAN TO GATHER NEEDED DATA: TOXICITY SENSOR TESTING TASK

Background. The U.S. Army Center for Environmental Health Research (USACEHR) is developing an Environmental Sentinel Biomonitor (ESB) system to provide rapid toxicity identification for a broad spectrum of chemicals in water. As part of a technology downselection effort, an expert panel has identified several toxicity sensors as candidates for inclusion in an ESB system. To help select the best toxicity sensors for use in an ESB system, USACEHR seeks to have these sensors tested against a common set of test chemicals.

Purpose. This task will provide essential toxicity response information to 15 chemicals (provided as blind samples) for one toxicity sensor.

Government-provided materials. Through a contractor, the Government will provide 15 chemicals to be tested (furnished as blind samples) and instructions for providing test data.

Task requirements. The following items are to be provided under this task.

1. A detailed protocol submitted for approval prior to testing initiation. Detailed instructions will be provided later, but, at a minimum, the protocol will include:

    a. Description of sample handling procedures.

    b. Test methodology to be followed, including QA/QC procedures.

    c. Exposure period, endpoints monitored, and statistical methods used to generate endpoint data. The endpoint value(s) must be generated for at least one exposure interval of 60 minutes or less, and the same exposure intervals must be used.

2. For each of the 15 blind samples received from the contractor:

    a. Conduct one range-finding test based on log dilutions of a stock solution made from the sample provided. The range-finding test will bracket the test concentrations to be used in the definitive tests.

    b. Conduct three separate definitive tests to establish the response endpoints defined in the protocols. At least five concentrations should be tested within the log interval determined in the range-find test. [If an endpoint response is not achieved using the undiluted blind sample in the range-find test, confirm this in a definitive test using only the blind sample concentration.]

    c. Prepare sample solutions to be used in testing by dilution in distilled water. If a defined culture medium is required for testing, a twice concentrated medium

d. should be diluted 50% with the test sample to be evaluated. If distilled water cannot be used as a diluent and a specific culture medium is not specified, reconstituted soft water medium may be used (see table below). Tap water or well water must not be used in sample preparation or testing.

e. Provide results from each test conducted, including endpoint responses and concentrations, exposure period(s), and applicable statistical confidence limits.

Deliverables: Approved test protocol for each toxicity sensor and completed test results for each toxicity sensor and chemical tested.

Recommended Composition of Soft Reconstituted Freshwater (if required)

Chemical	Concentration (mg/L)
$NaHCO_3$	48
$CaSO_4 \cdot 2\ H_2O$	30
$MgSO_4$	30
KCl	2.0

Characteristics: pH after aeration – 7.3 to 7.5; hardness (mg/L, as $CaCO_3$) – 40 to 48; alkalinity (mg/L, as $CaCO_3$) – 30 to 35.

Reference: Marking, L.L. and V.K. Dawson. 1973. Toxicity of quinaldine sulfate to fish. Investigations in Fish Control, No. 48, U.S. Fish and Wildlife Service, Washington, DC.