

USACEHR TECHNICAL REPORT 11-01

AN EVALUATION OF THE PCB TOX-SPOT WATER TOXICITY TEST



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**United States Army Center for Environmental Health Research
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14. ABSTRACT The United States Army Center for Environmental Health Research (USACEHR) is developing an Environmental Sentinel Biomonitor (ESB) system to test Army drinking water supplies for the presence of toxic industrial chemicals (TICs). One of the technologies considered for inclusion in the ESB system is the PCB TOX-SPOT Chemiluminescence Test, a rapid assay that measures changes in luminescence of the bacteria Photobacterium leiognathi as an indicator of toxicity. The TOX-SPOT test was able to respond to only 5 of 18 chemicals in a test set identified by an Army user group within a desired sensitivity range. Further, the TOX-SPOT kit has three reagents that must be stored at -14°C, which is undesirable for field use. Evaluation of TOX-SPOT reagents held under refrigeration at 6°C during a 12 month storage period produced inconsistent toxicity test results. Based on these test results, the TOX-SPOT test is not recommended for inclusion as part of the ESB system.					
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Table of Contents

1. Introduction	1
2. Materials and Methods	4
2.1 <i>TOX-SPOT Test Method</i>	4
2.2 <i>Inhibition Threshold Determination and Toxicant Testing</i>	5
2.3 <i>Reagent Stability Testing</i>	6
3. Results and Discussion	7
3.1 <i>Inhibition Threshold Determination</i>	7
3.2 <i>Toxicant Testing</i>	9
3.3 <i>Interference Testing</i>	12
3.4 <i>Reagent Stability Testing</i>	12
3.5 <i>Conclusions</i>	16
Acknowledgments	17
References	18
List of Abbreviations and Acronyms	20
Appendix A – PCB TOX-SPOT Procedure	21
Appendix B – Negative Control Statistics and Threshold Determinations	23
Appendix C – Chemicals Evaluated	34

List of Figures and Tables

Figures

2-1: The TOX-SPOT kit and carrying case.....	5
3-1: PCB TOX-SPOT – Millipore Water Testing.....	8
3-2: TOX-SPOT Negative Blank Samples Stored at 6°C	13
3-3: TOX-SPOT Toxicant Testing Samples Stored at 6°C.....	14
3-4: Negative Control Values of TOX-SPOT Refrigerated Reagents in Metal and Organic Buffers.....	15

Tables

Table 1-1: Estimated TOX-SPOT Detection Limits Compared to Military Exposure Guidelines and Human Lethal Concentration Values.....	3
Table 1-2: Potential Interferences.....	4
Table 3-1: TOX-SPOT Test Results.....	10
Table 3-2: Comparison of Two Luminescent Bacteria-based Technologies.....	11
Table 3-3: TOX-SPOT Interference Chemical Responses.....	12

1. Introduction

The United States Army Center for Environmental Health Research (USACEHR) has developed an Environmental Sentinel Biomonitor (ESB) system to test Army drinking water supplies for the presence of toxic industrial chemicals (TICs). For the first increment (Increment 1) of the ESB system, two toxicity sensor components were chosen: an Electrical Cell-substrate Impedance Sensing (ECIS) device and the Abraxis Organophosphate/Carbamate (OP/C) Screen pesticide assay. The acceptable response concentration range for each chemical selected by an Army user group (van der Schalie et al., 2006) was between the Military Exposure Guideline (MEG) concentration (based on consumption of 15 liters (L) per day of water for 7 – 14 days; USACHPPM, 2004) and the estimated human lethal concentration (HLC) (based on the consumption of 15 L of water per day for a 70 kilogram (kg) person; TERA, 2006). Together, the ECIS and OP/C assays responded to 14 out of 20 of the TICs within the MEG-HLC range.

The Increment 2 ESB system is intended for use with Army field water supplies at levels II and III of Army preventive medicine (PM) support in theaters of operation. For this application, improvements to the first increment are sought for responsiveness to chemicals and for reductions in the size and logistical requirements of the system. One candidate toxicity sensor for the increment 2 ESB system is the TOX-SPOT (or portable contamination biomonitor [PCB] TOX-SPOT), which may offer improvements in:

- **Chemical response** (vendor-supplied data show that TOX-SPOT responds similarly to the ECIS sensor by detecting at least 8 of 18 chemicals below the HLC, while also detecting a chemical that ECIS could not detect: cyanide);
- **Ruggedness** (portable design with no pumps, hoses, and few moving parts);
- **Time-to-results** (no pre-exposure period required, with test time reduced from 60 to 25 minutes); and
- **Sensor size** (available with a hand-held unit reader).

The TOX-SPOT is commercially available and is suitably small and lightweight (12 pounds) for the ESB Increment 2 system requirements. The TOX-SPOT contains a 30°C incubator, and its supplies and luminometer are fashioned to fit inside a manufacturer's briefcase-size case. The TOX-SPOT comes with a car adapter and 110/120 V power cord, and the luminometer is battery powered.

For optimal operation of the ESB system in the field, to control temperature and other environmental conditions should be minimized. Toxicity sensors with consumables requiring freezing are considered unacceptable, refrigeration of consumables, however, may be acceptable in many field situations. Level II forward operating bases (FOBs) have varying capabilities and equipment, but PM detachments at a FOB usually have access to a refrigerator. Currently, the TOX-SPOT assay requires several of its reagents and the test bacteria to be frozen, and a hydration buffer that must be refrigerated.

There are preliminary indications that TOX-SPOT reagents may remain stable and bacteria remain viable for extended periods of time when not frozen. TOX-SPOT responses to cadmium and parathion using frozen reagents were equivalent to responses obtained using reagents that were stored for up to 7 days at 25°C, and up to 5 days at 30°C (Ulitzur et al., 2002). It is not known how long the reagents might remain usable if

refrigerated instead of frozen. One goal of this study, therefore, was to determine whether TOX-SPOT reagents can be refrigerated (at 4-8°C) for up to 9 months or more without losing test functionality; nine months is the minimum target storage period for consumables intended for use in the ESB system. The stability of refrigerated reagents over time was determined using repeated tests with one organic toxicant (pentachlorophenate) and one inorganic toxicant (copper).

In addition to meeting reagent storage requirements for the ESB system, the TOX-SPOT must respond with appropriate sensitivity to the toxic effects of a broad range of chemicals. The 18 chemicals selected for ESB system testing by an Army user group included chemicals having varying modes of toxic action. Six “interference” chemicals were included as well to help ensure that candidate toxicity sensors would not respond to conditions that were harmful to human health. The MEG and HLC values for the 18 test chemicals are shown in Table 1-1; Table 1-2 shows the potential interference chemicals and their test concentrations. According to vendor-supplied data, the TOX-SPOT can detect 8 of 17 chemicals within the MEG-HLC range (Table 1-1); vendor data was not available for acrylonitrile or the interferences. Another goal of this study was to conduct range-finding toxicity tests to determine if TOX-SPOT responses to the full range of 18 chemicals and 6 interferences were consistent with the vendor data.

Since preliminary testing showed considerable control response variability, a third goal of this study was to re-evaluate the vendor-recommended 50% inhibition threshold for a toxic effect. Negative control TOX-SPOT data were analyzed statistically to determine the minimum inhibition threshold needed to support the Army’s requirement for a false positive rate of less than 0.001 (Appendix B). The statistically-determined inhibition threshold was then used in all toxicity studies conducted.

Table 1-1: Estimated TOX-SPOT Detection Limits Compared to Military Exposure Guidelines and Human Lethal Concentration Values			
Test Chemicals^a	MEG^b (mg/L)	HLC^c (mg/L)	TOX-SPOT Detection Limit^d
Acrylonitrile	0.47	4.2	Not tested
Aldicarb	0.0047	0.17	95
Ammonia	30	924	>1000(NH ₄ Cl)
Arsenic (sodium arsenite)	0.02	4.5	3.5
Azide (sodium azide)	0.12	47	3.6
Copper (sulfate)	0.047	103	1.5
Cyanide (sodium)	2	14	0.25
Fenamiphos	0.0042	0.56	0.95
Fluoroacetate (sodium)	0.00072	5.1	50
Mercury (chloride)	0.01	24.7	0.05
Methamidophos	0.00023	1.4	>1000
Methyl parathion	0.14	33.6	5
Nicotine	0.13	16.8	1000
Paraquat (dichloride)	0.034	4.6	>1000
Pentachlorophenate (sodium)	0.14	71.9	0.01
Phenol	2.8	91.5	217
Thallium (sulfate)	0.0033	13.5	112
Toluene	9.3	840	200
Legend		Number of Chemicals	
	Chemical detected in MEG-HLC range	6	
	Chemical detected below MEG	2	
	Chemical detected above HLC	6	
	Chemical not detected	4	

^a More information on chemicals available in Appendix D

^b **MEG** – 7 to 14 day Military Exposure Guidelines (70 kg person, 15 liter [L]/day consumption), when available; 1 year MEG for copper, fluoroacetate, and strychnine; < 7 day MEG for nicotine; fenamiphos MEG estimated from terbufos (Richards, personal communication)

^c **HLC** – Human Lethal Concentration (70 kg person, 15 L/day consumption)

^d Nirit Ulitzur, CheckLight Ltd., 5 Mar 2010 (personal communication)

Table 1-2: Potential Interferences	
Test Chemicals	Concentration (mg/L)
Chlorine	10
Chloramines	10
Geosmin	0.0001
Methyl-isoborneol (MIB)	0.0001
Humic / Fulvic Acids (50%/50%)	5 (2.5/2.5)
Hard Water	250

2. Materials and Methods

2.1. TOX-SPOT Test Method

The TOX-SPOT assay uses naturally bioluminescent *Photobacterium leiognathi* (Katznelson and Ulitzur, 1977) and is commercially available through Checklight Ltd, (Figure 2-1). The premise of the assay is that the inhibition of bacterial light output is an indication of the presence of a toxic chemical. The bacterial reagent is stored at -20°C and is removed immediately prior to testing. The assay has three types of buffered reagents: an organic buffer series designed to aid detection of organic contaminants in samples, a metal buffer series to improve metal detection, and a hydration buffer to reconstitute the bacteria. Each series contains 4 vials: a negative control (NC), positive control (PC), and duplicate sample vials (S1 and S2). Each organic PC vial contains 0.08 µg chloroacetate and each metal PC contains 0.015 µg copper sulfate (80 mg/L chloroacetate and 6.4 mg/L copper, respectively, when reconstituted). The S1 and S2 vials, to which water samples to be tested are added, contain only the proprietary salt buffers that are consistent with the negative control vials. Thus, each TOX-SPOT test can evaluate two water samples for both organic and metal toxicants. The water samples are incubated in the vials with the buffered reagents for 10 minutes at 30°C. The bacterial reagent is then placed into each vial and incubated for 15 minutes. The vials are then individually read using a hand-held luminometer that measures the light intensity of the sample in arbitrary light intensity units. Chemicals that interfere with the bacteria cause a decreased light intensity output. This output is compared to the appropriate negative control buffer, thereby generating a percent (%) inhibition.

The following materials were purchased from Checklight Ltd. (Box 72 Qiryat-Tiv'on 36000, Israel) for TOX-SPOT testing:

- SPOT-01-R (refill kit): biosensor vials, Pro-Metal Buffer Vials, Pro-Organic Buffer Vials, Pro-Metal Positive Control Vials Pro-Organic Positive Control Vials, Hydration buffer, empty 50 ml centrifuge tubes, empty luminometer tubes and caps
- Kikkoman portable luminometer
- Test Kit (carrying case, incubator, incubator power adapter, instructions, pipettor, disposable tips)

Other materials used in testing were:

- Thermo Orion Precision Low Temperature Model 818 incubator
- ERTCO CAT#R-020 refrigerator thermometer (ethylene glycol bottle liquid)
- Veriteq temperature data-logger, model SP-1000-21N
- Nissin N-20M vortex mixer, AR Brown Co., LTD, Tokyo, Japan
- Millipore Ultrapure Water System (Milli-Que Gradient)



Figure 2-1. The TOX-SPOT kit and its carrying case.

2.2 Inhibition Threshold Determination and Toxicant Testing

The test endpoint is the percent inhibition when compared to a control (same buffer). The manufacturer recommends that an inhibition or stimulation light output of 50% or greater be considered a “detect.” However, given the wide variation in stimulation observed in negative control data (between +42% (inhibition) and -73% (stimulation)), the level of inhibition required was evaluated statistically rather than being automatically set at the manufacturer’s recommendation of 50%.

The threshold inhibition concentration was determined based on an analysis of negative control variability. Light inhibition levels were measured for 30 control metal buffer solutions and 30 control organic buffer solutions. These data were then analyzed

statistically, as described in Appendix B. Evaluations included basic distributional properties, bias between the (negative) control and “unknowns” (control samples 1 and 2), variance of the control-unknown differences, correlation between sample 1 and sample 2 controls and other measures, relation of control-unknown differences to control-unknown means, and determining the threshold for identifying a toxic response.

Range find toxicity testing was completed by testing two samples per buffer (S1 and S2) for each chemical at the human lethal concentration. If the chemical was not detected, two more samples were tested at 10 times the HLC of that chemical; then at the chemical stock concentration if there was no detection at 10 times the HLC. If the chemical was detected, further testing at lower concentrations was completed if stocks were available. Further testing with ammonia, mercury, copper, and toluene were not completed.

Chemical stock solutions were prepared in American Society of Testing and Materials (ASTM) Type II water, also referred to as Millipore water or negative blank water in this report. Pentachlorophenate (PCP) (sodium) was prepared by titrating pentachlorophenol in 50 millimolar (mM) phosphate buffer with 1 molar hydrochloric acid to pH 7.5. Test chemicals were used either the same day as analyzed (when possible) or within two weeks when held at 4°C storage. All test chemicals were verified as stable for two weeks under these storage conditions. Volatile chemicals (acrylonitrile and toluene) were stored in zero headspace vials at 4°C. Two test chemicals (fenamiphos, and methamidophos) and three interference chemicals (geosmin, humic/fulvic acids, and methyl-isoborneol) were tested at nominal concentrations because suitable methods for analysis at the required concentrations were not available. All other stock concentrations of test compounds were analyzed using the analytical methods indicated in Appendix C. The recommended pH range for the assay was 6-8.5, (Nirit Ulitzur, e-mail communication), so stock solutions were titrated (if necessary) with either 1N HCl or 1N NaOH to meet the requirements of the pH range.

2.3 Reagent Stability Testing

To determine how long valid TOX-SPOT test results could be obtained using refrigerated reagents, the organic buffer, metal buffer, and bacterial reagent vials were placed in a 6 °C incubator. At regular intervals, sets of these reagents (organic NC, PC, S1, and S2 and metal NC, PC, S1 and S2) were removed for testing. One set was used for negative blank sample testing (S1 and S2 were Millipore water samples) and another set was used for reference toxicity (S1 = 0.1 mg/L PCP and S2 = 1.5 mg/L copper) for each time interval. Testing occurred on 0 (initial) 7, 14, 21, 38, 60, 90, 180, 270, and 365 days after refrigeration.

Refrigerated reagents were stored at 6°C to correspond to the storage temperature of other ESB system components. A Thermo-Orion Precision Low Temperature Model 818 incubator was used. Temperature recorders showed good temperature stability, with a mean of 5.99°C, and a standard deviation of 0.096 over the test period. A manual, National Institute of Standards and Technology traceable refrigerator thermometer (ERTCO CAT#R-020) verified the incubator temperature.

3. Results and Discussion

3.1 Inhibition Threshold Determination

All 30 negative control samples tested were below the 50% inhibition detection threshold for both the metal and organic buffers (Figure 3-1). Variability was greater for stimulation, with 3 samples exceeding 50% stimulation (two in metal buffer; one in organic buffer). Based on the statistical analysis (Appendix B), light inhibition measurements were normally distributed, with the exception of one outlier data point from the organic buffer data (sample 28 in Figure 3-1). Using a test for dependence within metals and organics values further reinforced the need for including a negative control in each test. There was some correlation between the two samples in each test. The metal buffer data indicated a negative bias, meaning that the sample readings tended to be higher than the negative control, which could increase the possibility of false negative results. It is unclear whether this is a real effect, since the same trend was not observed for the organic buffer data. Although all 30 control samples were negative, statistical probabilities based upon percent inhibition results show that for both the metal and the organic buffers, the false positive rate exceeds 1 in 100 for response threshold of 50% inhibition. Given the tendency for greater variation in the negative controls in stimulation than in inhibition, we chose a one-tailed indicator of response (inhibition) for the endpoint. For this metric, the analysis showed that the response threshold should be raised to 75% inhibition to obtain a false positive protection rate of 1 in 1000, as dictated by the Army. The 75% inhibition detection level was used for all TOX-SPOT tests conducted in this study.

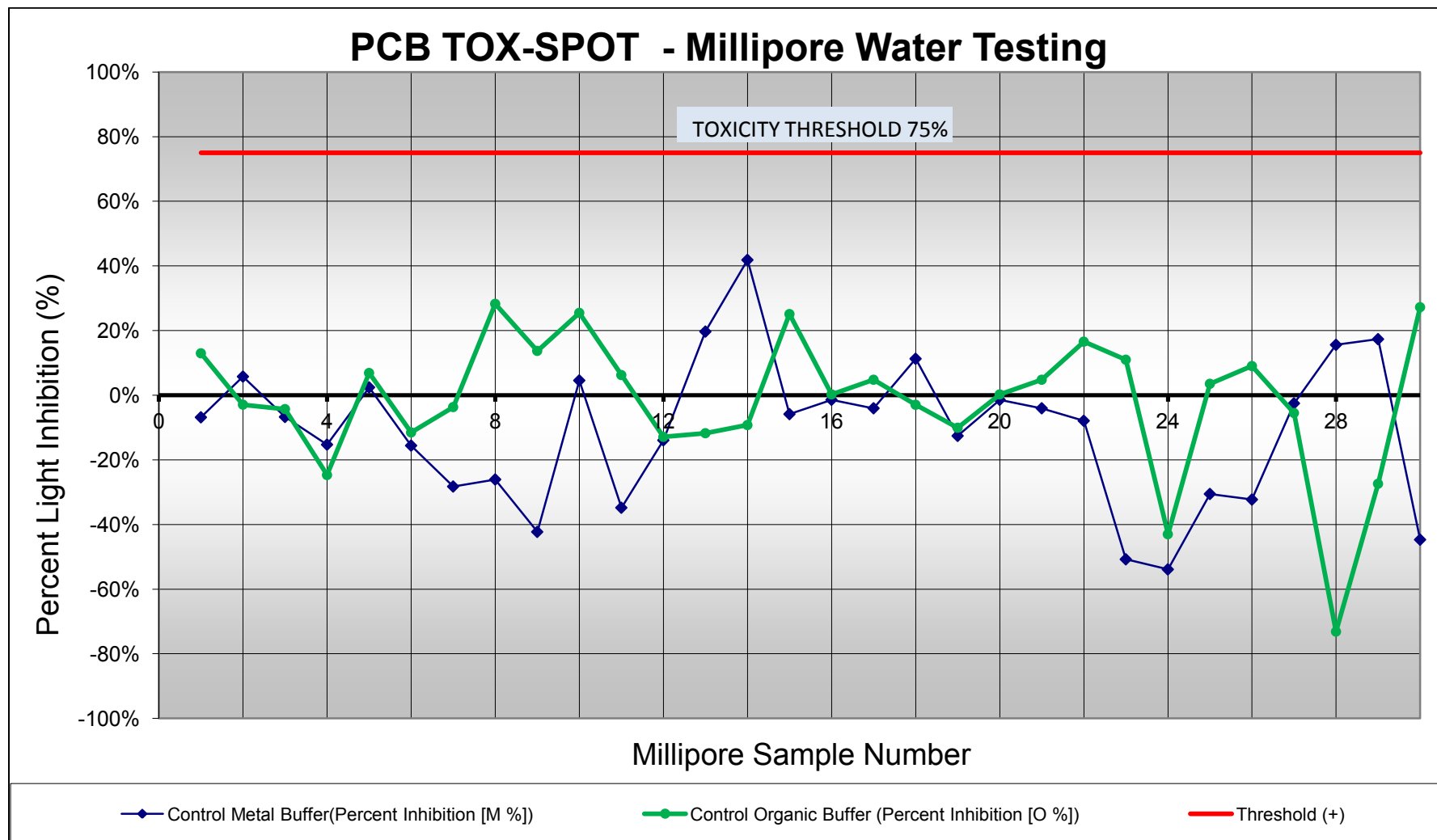


Figure 3-1. The TOX-SPOT relative percent (%) light inhibition of negative blank samples (n=30).

3.2 Toxicant Testing

All chemical testing was completed with reagents stored according to manufacturer recommendations (bacteria and metal and organic buffers at -20°C, and hydration buffer at 4°C).

Using the 75% inhibition threshold (false positive rate of 0.001), the TOX-SPOT assay detected 5 chemicals in the MEG-HLC range (azide, copper, mercury, methyl parathion and toluene) and 2 chemicals below the MEG (cyanide and PCP). The remaining 11 chemicals were detected either above the HLC or not at all (Table 3-1). The results were consistent with vendor-supplied data except for arsenic (response found at a concentration 10 times higher than the vendor estimate).

With the vendor-recommended 50% threshold applied, TOX-SPOT would also detect ammonia at the HLC. Phenol would be detected, although above the HLC. Nicotine would be detected with >50% stimulation (-94% inhibition). Thus, TOX-SPOT would detect 7 of the 18 chemicals in the MEG-HLC range, 2 below the MEG-HLC range, and 9 above the HLC or not detected at all.

In Table 3-2, TOX-SPOT detection data is compared to data from the Microtox toxicity test, which uses luminescent bacteria as the biosensor. Microtox was tested in the downselection process for the Increment I version of the ESB (Widder et al., 2007). TOX-SPOT and Microtox both responded to copper, mercury, methyl parathion, PCP, and toluene in the MEG-HLC range. Additionally, TOX-SPOT detected ammonia and azide. Azide was not tested in Microtox assays in Widder et al., (2007) but a Microtox response of 400 mg/L for azide was reported by Chang et al., 1981. Microtox was more sensitive to phenol than TOX-SPOT, and was less sensitive to PCP, responding to both in the MEG-HLC range. For chemicals detected above the HLC (such as acrylonitrile, arsenic, fenamiphos, nicotine, paraquat and thallium), the MDL for both technologies were similar, except that TOX-SPOT was 20 times more sensitive to fluoroacetate. In terms of overall toxicant sensitivity, there was not a marked advantage to using TOX-SPOT over Microtox.

Table 3-1: TOX-SPOT Test Results						
Test Chemicals	MEG ^{a,b}	Vendor Detection Limit ^b (Exceeds 50% Stimulation/ Inhibition)	USACEHR Estimated Detection Limit (Exceeds 75% Inhibition Only)			HLC ^{b,e}
			Concentration (mg/L) ^b	Mean % Inhibition Organic ^{c,d}	Mean % Inhibition Metal ^{c,d}	
Acrylonitrile	0.47	Not tested	4.2-1140	5, 41	12, 80 ^f	4.2
Aldicarb	0.0047	95	>95	30	4	0.17
Ammonia	30	>262	>.924 as N	59	13	924
Arsenic (sodium arsenite)	0.02	3.5	4.5-35	28, 100	33, 94	4.5
Azide (sodium)	0.12	3.6	10	76	9	47
Copper (sulfate)	0.047	1.5	<1.5	98	-10	103
Cyanide (sodium)	2	0.25	< 2	100	-33	14
Fenamiphos	0.0042	0.95	0.56 - 5.6	20, 80	14, -2	0.56
Fluoroacetate (sodium)	0.00072	50	3.9-50	6, 78	1, -41	5.1
Mercury	0.01	0.05	<24.7	100	100	24.7
Methamidophos	0.00023	>1000	>1000	-3	-1.5	1.4
Methyl parathion	0.14	5	5-33.6	90	45	33.6
Nicotine	0.13	1000	>16.8	-94	13	16.8
Paraquat (dichloride)	0.034	>1000	944	24	70	4.6
Pentachlorophenate (sodium)	0.14	0.01	0.01 - 0.1	18, 100	-28, -18	71.9
Phenol	2.8	217	214	50	37	91.5
Thallium (sulfate)	0.0033	112	115-1000	65	90	13.5
Toluene	9.3	200	<227	89	-6	840
Legend				TOX-SPOT Summary		
	Chemical detected in MEG-HLC range ($\geq 50\%$)			5		
	Chemical detected below MEG			2		
	Chemical detected above HLC			5		
	Chemical not detected at any concentration tested			6		

^a MEG – 7 to 14 day Military Exposure Guidelines (15 L/day), when available, 1 year MEG for copper, fluoroacetate; < 7 day MEG for nicotine; fenamiphos MEG estimated from terbufos (Richards, personal communication),

^b mg/L – liter,

^c n = 2 samples

^d non-detect %, followed by detect %,

^e HLC – Human Lethal Concentration (70 kg person, 15 L/day)

^f red color indicates chemical was detected above 75% inhibition threshold

Table 3-2: Comparison of Two Luminescent Bacteria-based Technologies				
Test Chemicals	MEG^{a,b}	TOX-SPOT Estimated Detection Limit^b (Exceeds 75% Inhibition Only)	Microtox EC50^{b,c}	HLC^{b,d}
Acrylonitrile	0.47	4.2-1140	>600	4.2
Aldicarb	0.0047	>95	32.1	0.17
Ammonia	30	>924 as N	1490	924
Arsenic (sodium arsenite)	0.02	4.5-35	15.2	4.5
Azide (sodium)	0.12	10	Not tested	47
Copper (sulfate)	0.047	<1.5	0.21	103
Cyanide (sodium)	2	< 2	1.6	14
Fenamiphos	0.0042	0.56 - 5.6	24.2	0.56
Fluoroacetate (sodium)	0.00072	3.9-50	1989	5.1
Mercury	0.01	<24.7	0.14	24.7
Methamidophos	0.00023	>1000	>239	1.4
Methyl parathion	0.14	5-33.6	0.85	33.6
Nicotine	0.13	>16.8	69	16.8
Paraquat (dichloride)	0.034	944	1136	4.6
Pentachlorophenate (sodium)	0.14	0.01 - 0.1	1.2	71.9
Phenol	2.8	214	29.6	91.5
Thallium (sulfate)	0.0033	115-1000	>500	13.5
Toluene	9.3	<227 ^e	14.8	840
Legend		TOX-SPOT	Microtox	
	Chemical detected in MEG-HLC range	5	6	
	Chemical detected below MEG	2	1	
	Chemical detected above HLC	5	7	
	Chemical not detected	6	3	

^a **MEG** – 7 to 14 day Military Exposure Guidelines (15 L/day), when available, 1 year MEG for copper, fluoroacetate, and strychnine; < 7 day MEG for nicotine; fenamiphos MEG estimated from terbufos (Richards, personal communication),

^b mg/L

^c Data from FOUO Widder et al., May 2007

^d **HLC** – Human Lethal Concentration (70 kg person, 15 L/day)

^e Toluene stock made at solubility limit

3.3 Interference Testing

It is unlikely that the TOX-SPOT will show light inhibition in response to cyanobacterial byproducts (geosmin and MIB), water hardness, or humic and fulvic acids if present in source or product drinking waters at the levels tested in this study (Table 3-3). The degree of stimulation shown in response to hard water is another reason not to use stimulation as an indication of toxicity. TOX-SPOT is strongly affected by 10 mg/L of chlorine or chloramine, but it is possible to dechlorinate a sample by adding a mild reducing agent such as sodium bisulfite or sodium thiosulfate. Issues involved with dechlorinating a water sample prior to testing the sample with a toxicity sensor have been previously described (Trader et al., 2010, and van der Schalie et al., 2005).

Table 3-3: TOX-SPOT Interference Chemical Responses				
Test Chemicals	Concentration (mg/L)	Response*	Mean Percent Inhibition (%)	
			Organic Buffer	Metal Buffer
Chlorine	10	2 of 2	100**	100
Chloramines	10	2 of 2	100	100
Geosmin	0.0001	0 of 3	7	-21
Methyl-isoborneol (MIB)	0.0001	0 of 3	-12	-25
Humic / Fulvic Acids (50%/50%)	5 (2.5/2.5)	0 of 3	-1	-7
Hard Water	250	0 of 3	-46	-101

* A response is an inhibition of 75% or greater in the respective buffer's negative control

** red color indicates chemical was detected above 75% inhibition threshold

3.4 Refrigerated Reagents Testing

The potential for long-term storage of refrigerated reagents was evaluated by determining both the response levels and reproducibility of negative controls, and the consistency of toxicant detection. Negative blank samples gave no detection above 75% at any time point (See figure 3-2). The metal buffer samples, however, tended to show increasing light stimulation as time increased. If the manufacturer's recommendation of a 50% change (as either inhibition or stimulation) detection threshold is applied, samples at 2 weeks, 6 months, 9 months and 12 months would not be suitable for testing. The negative controls (to which the samples were compared) were the same negative blank water sample used in the S1 and S2 samples. It is unclear why there is a decreasing trend with time, since all bacteria used at each time interval came from the same vial.

Copper was detected at all time points (Figure 3-3). PCP was not detected at the 6 month mark, but was detected at 9 and 12 months. The raw light units (RLU) of negative blank samples in metal buffers were much higher than negative blank samples in organic buffers (see Figure 3-4). The RLU of samples trended downward over time in both

buffers. Since the manufacturer recommends that bacterial reagents be stored at -20°C, it is possible that bacterial viability decreased with time, leading to lower light output.

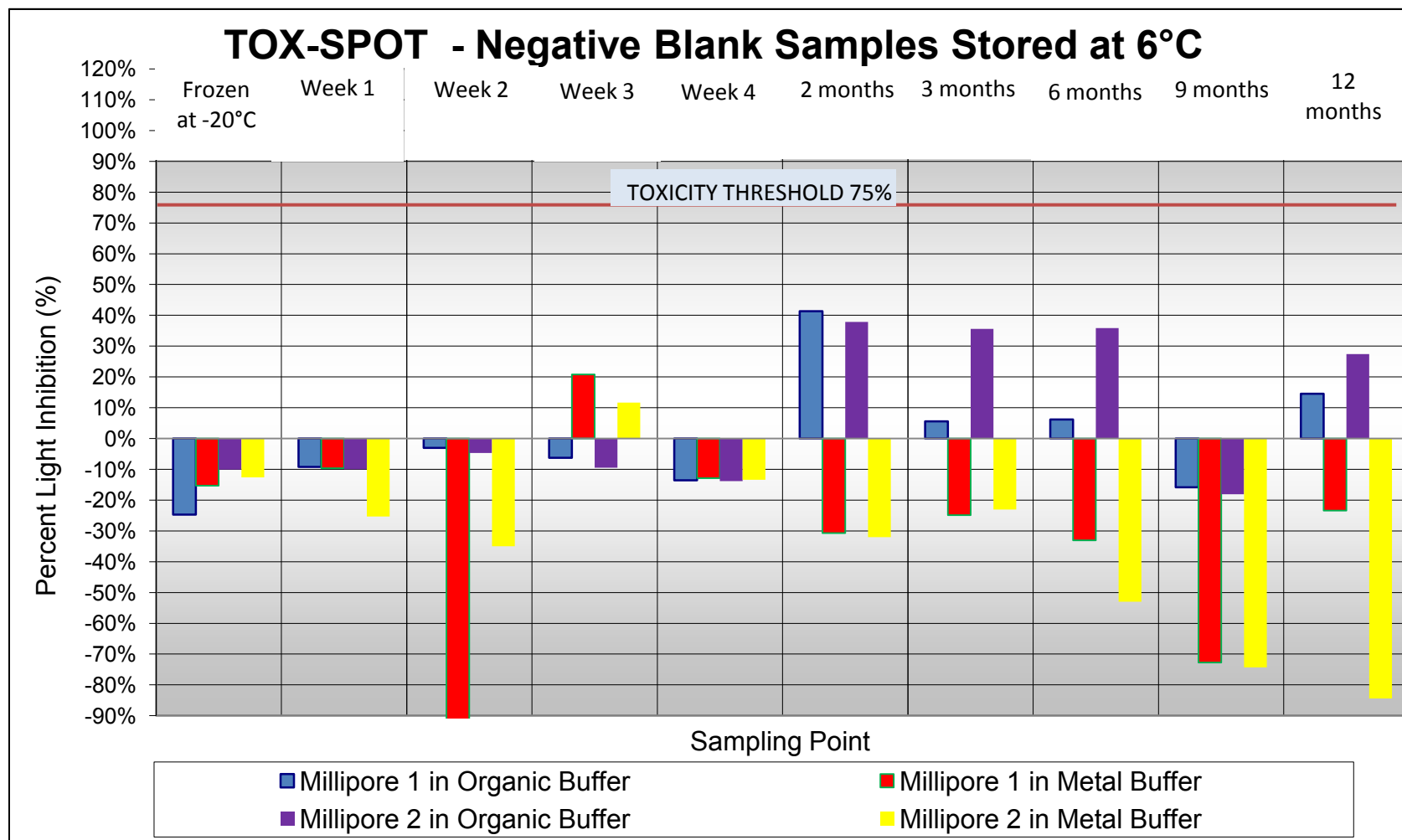


Figure 3-2. Long-term testing of 6°C reagents with negative blank water samples in both organic and metal buffers. *The negative control reading in the metal buffer was low, hence all other metal readings were highly negative (Week 2, Millipore 1 sample in metal value was -122%, truncated for viewing purposes).

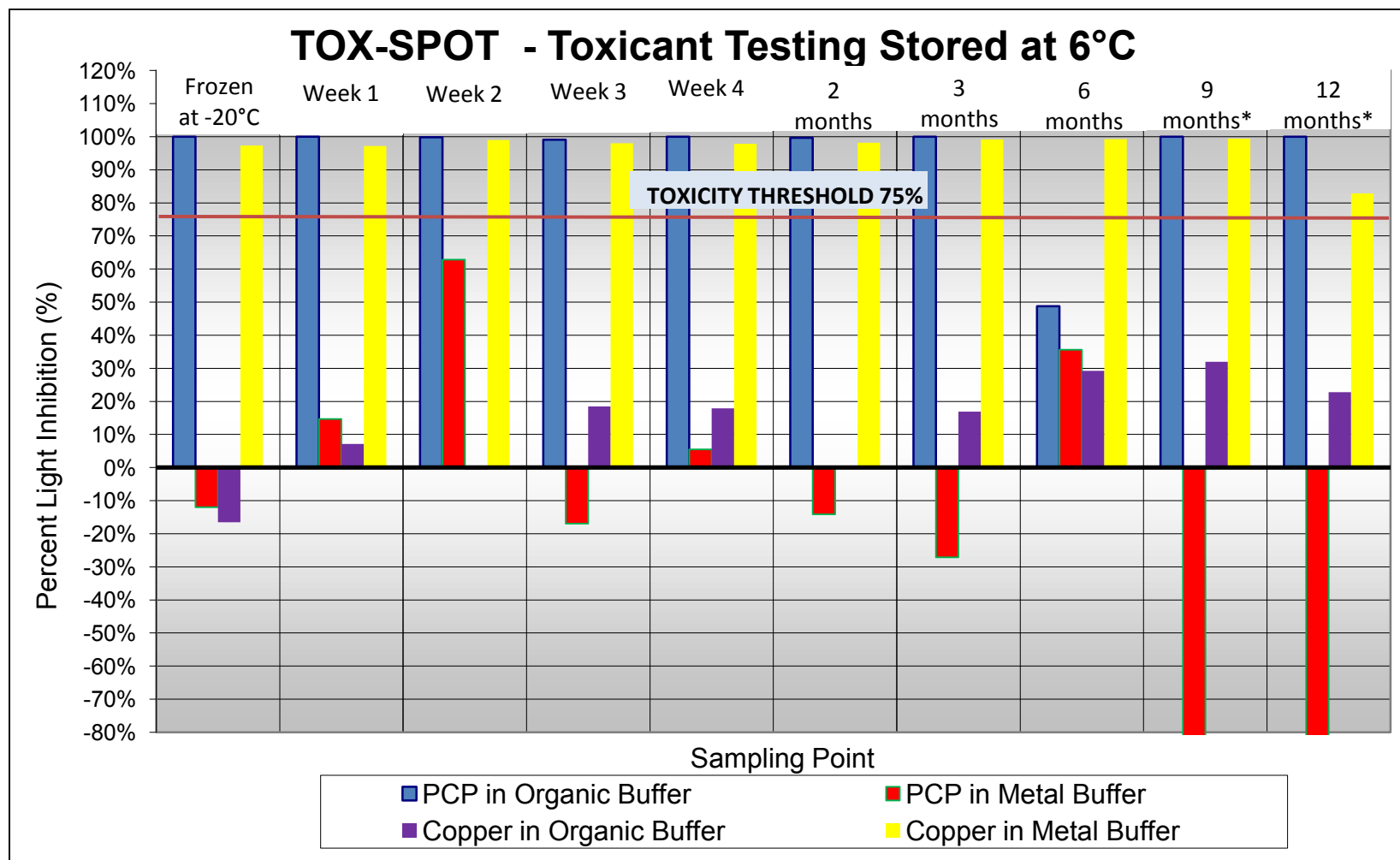


Figure 3-3. Long-term testing of 6°C reagents with pentachlorophenol (0.1 mg/L) and copper sulfate (1.5 mg/L) in both organic and metal buffers. * Response to PCP in the metal buffer at the 9 month time point was -148%; -101% at the 12 month time point; the graph is truncated.

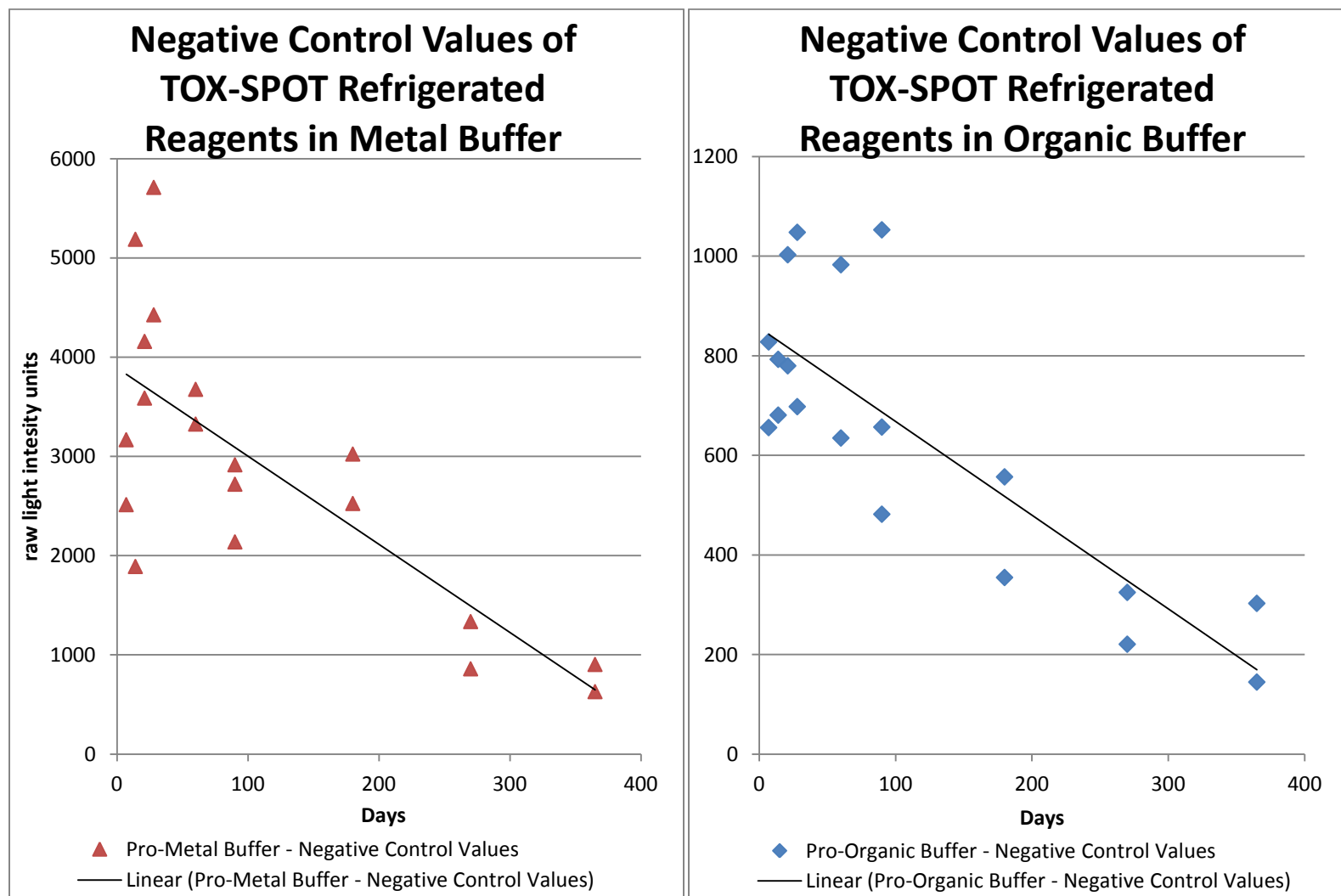


Figure 3-4. Raw relative light intensities for control metal (left) and organic (right) buffers.

3.5 Conclusions

The PCB TOX-SPOT Chemiluminescence Test is a self-contained kit with sturdy packaging and materials, and results are ready within 30 minutes. Based on testing conducted in this study, the kit can detect 7 of 18 chemicals below the HLC. The TOX-SPOT has better detection sensitivity for azide, cyanide, and PCP than the current ESB Increment 2 sensors (although cyanide and PCP are detected below the MEG), but was less sensitive for the other 15 chemicals. The TOX-SPOT does not have significant advantages in detection capability over Microtox; TOX-SPOT can detect 5 of 18 chemicals in the MEG-HLC range, while Microtox detects 6 of 18 chemicals. Their overall chemical sensitivity is generally similar.

The TOX-SPOT kit, as designed, requires 2 different storage temperatures; 3 reagents (bacteria, organic and metal buffers) at -14°C, and the hydration buffer at 4°C. If the bacteria and organic/metal buffers could be stored in refrigeration, the kit would require one less piece of equipment (freezer) and might be suitable for Army field use. However, our shelf-life data indicated that the TOX-SPOT reagents had inconsistent toxicant response during a 12 month storage period at 6°C, so further evaluation of this kit for Army field water tests is not recommended.

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References

Chang, J.C., Taylor, P.B., and F.R. Leach. 1981. Use of the Microtox Assay system for environmental samples. *Bulletin of Environmental Contamination and Toxicology*. 26 (1) : 150-156.

Katznelson R, Ulitzur S. 1977. Control of luciferase synthesis in a newly isolated strain of *Photobacterium leiognathi*. *Arch Microbiol.*, Dec 15;115(3):347-51.

Toxicology Excellence for Risk Assessment (TERA). 2006. Derivation of Human Lethal Doses. USACEHR Technical Report 0802, U.S. Army Center for Environmental Health Research, Fort Detrick, MD. ADA494706.

Trader, D. E., and W. H. van der Schalie. 2010. An Evaluation of the Eclox Chemiluminescence Test, Hach Pesticide/Nerve Agent Test Strips, and Agri-Screen Test Tickets. USACEHR-TR-1002. Defense Technical Information Center Accession Number: ADA525515. U.S. Army Center for Environmental Health Research, Fort Detrick, MD.

Ulitzur, S., Lahav, T. and Ulitzur, N. 2002, A novel and sensitive test for rapid determination of water toxicity. *Environmental Toxicology*, 17: 291–296.
doi: 10.1002/tox.10060.

U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), Version 1.3—Updated May 2003 with January 2004 Addendum. Chemical Exposure Guidelines for Deployed Military Personnel. Technical Guide (TG) 230. U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground, MD. (<http://chppm-www.apgea.army.mil/documents/TG/TECHGUID/TG230.pdf>)

van der Schalie, W.H., R.R. James, and T.P. Gargan, II. 2006. Selection of a Battery of Rapid Toxicity Sensors for Drinking Water Evaluation. *Biosensors and Bioelectronics* 22:18-27.

van der Schalie, W.H., D.E. Trader Widder, M.W., T.R. Shedd and L.M. Brennan. 2005. A Residual Chlorine Removal Method to Allow Drinking Water Monitoring by Biological Early Warning Systems. USACEHR Technical Report 0501, ADA432455, U.S. Army Center for Environmental Health Research, Fort Detrick, MD.

Widder, M.W., L.M. Brennan, D.E. Trader, and W.H. van der Schalie. 2007. Rapid Toxicity Sensor Responses to Selected Chemicals in Water. USACEHR Technical Report 0701, ADB327259, U.S. Army Center for Environmental Health Research, Fort Detrick, MD.

List of Abbreviations and Acronyms

%	Percent
ASTM	American Society of Testing and Materials
°C	degree Celsius
ECIS	Electric Cell-substrate Impedance Sensing
ESB	Environmental Sentinel Biomonitor
FOB	Forward Operating Base
HLC	human lethal concentration
kg	kilogram
JCBRAWM	Joint Chemical Biological Radiological Agent Water Monitor
L	liter
M	Molar
MDL	minimum detection limit
MEG	Military Exposure Guidelines
mg	milligram
min	minute
MIB	methyl iso-borneol
mM	millimolar
NC	negative control
OP/C	organophosphate and carbamate
PC	positive control
PCB	portable contamination biomonitor
PCP	pentachlorophenate

PM	preventive medicine
RLU	relative light unit
TICs	Toxic Industrial Chemicals
USACEHR	U.S. Army Center for Environmental Health Research
USACHPPM	U.S. Center for Health Promotion and Preventative Medicine

Appendix A

PCB TOX-SPOT Procedure

Summarized Procedure for the PCB TOX-SPOT

A complete procedure and manual for the PCB-TOX SPOT is available at the following website:

<http://www.checklight.biz>

Preparation:

1. Turn on the portable incubator, ensuring it is set to 30°C.
2. Place 8 plastic tubes in the incubator to warm.
3. Remove the one vial of bacteria and 8 reagent vials (4 organic and 4 metal [negative control, positive control, Sample 1 and Sample 2 for each]) from -20°C, and the hydration buffer from the refrigerator.

Incubate Bacteria and Sample:

4. Place 0.9 ml of the hydration buffer into the bacteria, vortex the vial, and place in the incubator for 10 minutes.
5. Add 0.9 ml of blank sample water (Millipore water) to both negative and both positive control vials. Place 0.9 ml of sample 1 into both S1 vials, and likewise for sample 2 (S2). Vortex each vial.
6. After 10 minutes, place 0.1 ml of the bacteria into each of the 8 vials.
7. Vortex, and transfer each vial to the appropriate pre-warmed, plastic tube in the incubator by pouring directly from the glass vial to the plastic tube. Replace tubes back into the incubator for 15 minutes.

Measure the Sample:

8. Turn on the luminometer, and allow for the automatic 10 second calibration with the lid closed.
9. Using the orange tube holders, pick up each tube, and place into the luminometer. Press ENTER to measure the intensity.
10. Record the intensity values for each vial and compare the sample values to the respective buffer control.



Appendix B

Negative Control Statistics and Threshold Determination

Summary of Control-Control study for TOX-SPOT Water Testing System**Elgin Perry****eperry@chesapeake.net****6-24-2011**

This report focuses on the control-control response for percent inhibition measure for organic and metal buffers of the TOX-SPOT assay.

For these measures, the issues addressed in this assessment include: basic distributional properties, bias between control and unknown, variance of the control-unknown difference, correlation between sample 1 and sample 2 and other measures, relation of control-unknown difference to control-unknown mean, and threshold for identifying a toxic response.

Correlation Among Measures

Even after adjusting for the control by division (pct) or subtraction (dif), there appears to be significant correlations between Sample 1 and Sample 2.

Table 1. Correlations among assessments

Buffer	Variable 1	Variable 2	Correlation	p-value
Metal	S1	S2	0.7887	5e-04
	NC	S1	0.7621	0.001
	NC	S2	0.6735	0.0059
	S1pct	S2pct	0.6628	0.0071
	S1dif	S2dif	0.6143	0.0148
Organic	S1	S2	0.6162	0.0144
	NC	S1	0.6908	0.0044
	NC	S2	0.5923	0.02
	S1pct	S2pct	0.4408	0.1001
	S1dif	S2dif	0.4463	0.0954
M vs. O	M-NC	O-NC	-0.0312	0.9121
	M-S1	O-S1	-0.0773	0.7841
	M-S2	O-S2	-0.0687	0.8077

There is fairly strong test to test dependence within metal and organic measures. All measures taken in the test (NC, S1, S2) have positive correlation so that if the NC is high, it is likely that S1 and S2 will be high as well. On the other hand, there appears to be little correlation between metal and organic readings that use bacteria from the same vial.

Graphical Analysis of difference between Control and Unknown

The measure of importance is the percent inhibition of light production by the bacteria. Because these are control-control replicates, it is expected that this inhibition measure represents only random variability of the testing process. There should be no consistent bias between control and unknown readings because both receive the same treatment. Furthermore we expect that repeated tests through time should exhibit independence.

First, we graphically examine the basic distribution properties of percent inhibition for the metal buffer (Figure 1) and the organic buffer (Figure 2) and the same properties of a simple difference measure (control - sample) for the metal buffer (Figure 3) and organic buffer (Figure 4).

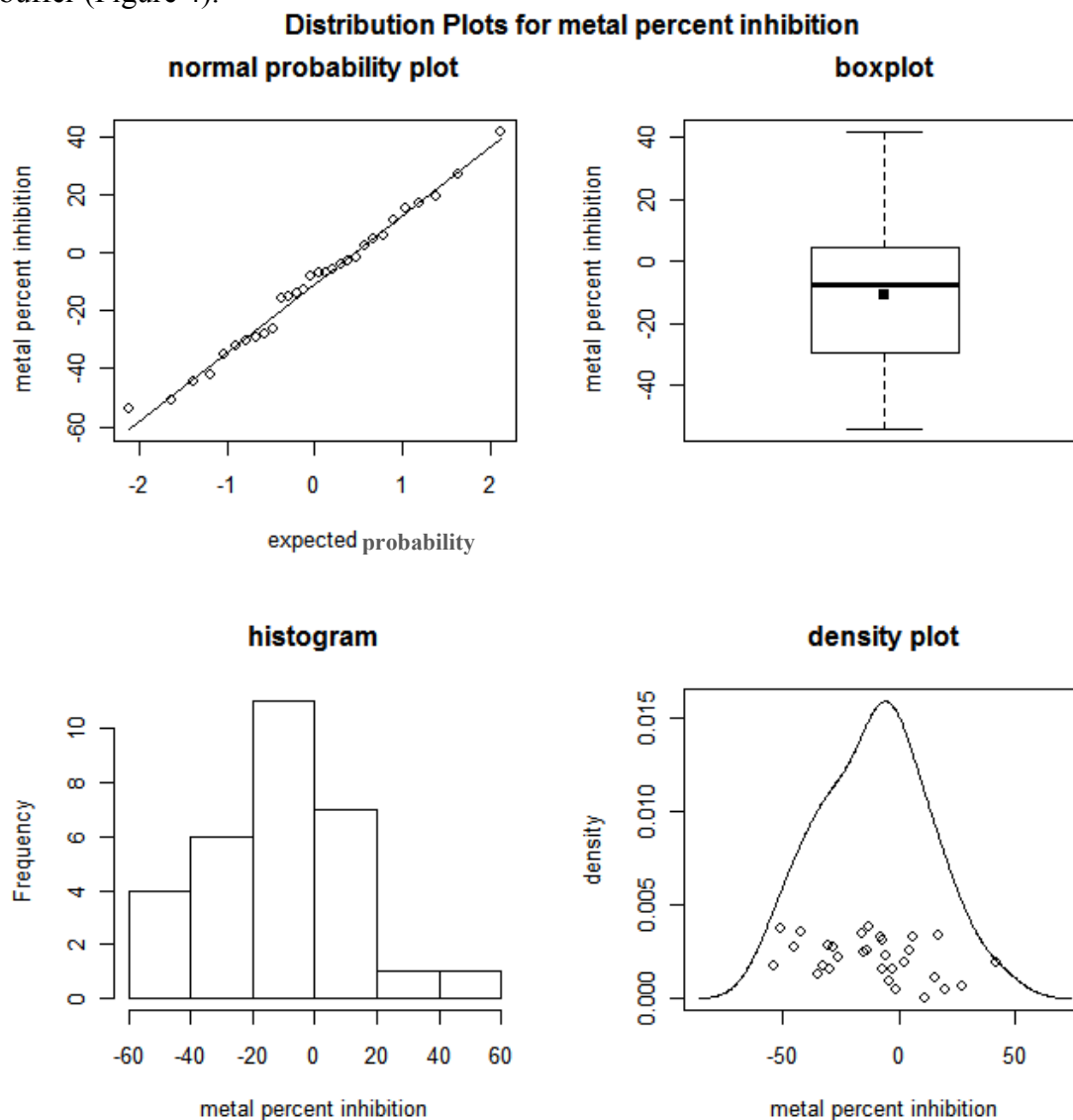


Figure 1. Distribution plots for the percent inhibition for samples 1 and 2 for metal buffer.

In the normal probability plot for metal percent inhibition (Figure 1), the data align along the diagonal line, which indicates that the innovations are well approximated by a normal

distribution. The symmetry of the remaining plots also confirms this normality property. Note that in the box plot, the histogram and the density plot it appears that the ratio data are centered slightly below zero indicating slight bias.

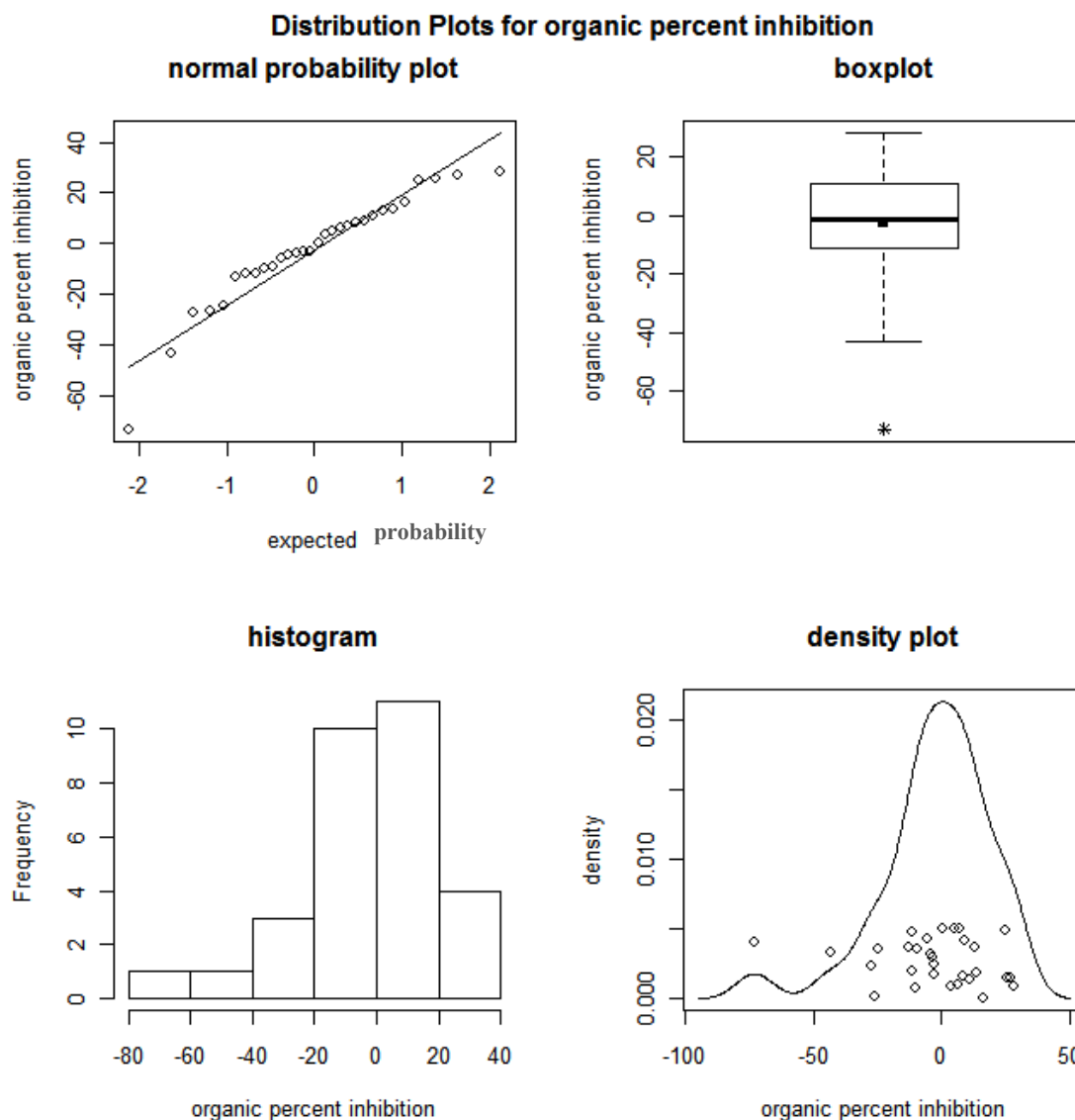


Figure 2. Distribution plots for the percent inhibition for samples 1 and 2 for organic buffer.

In the normal probability plot for organic percent inhibition (Figure 2), the data align fairly well along the diagonal line with the exception of one point which seems to be an extreme in a negative direction or a negative outlier when compared to expectation from the normal distribution. This outlier introduces an asymmetry of the remaining plots making the data appear skewed to the left. Other than this one point, the box plot, the histogram, and the density plot appear to be centered on zero as is expected for a control-control assessment. This outlier occurs in sample 2 of test 13 and has a percent inhibition of -73.14.

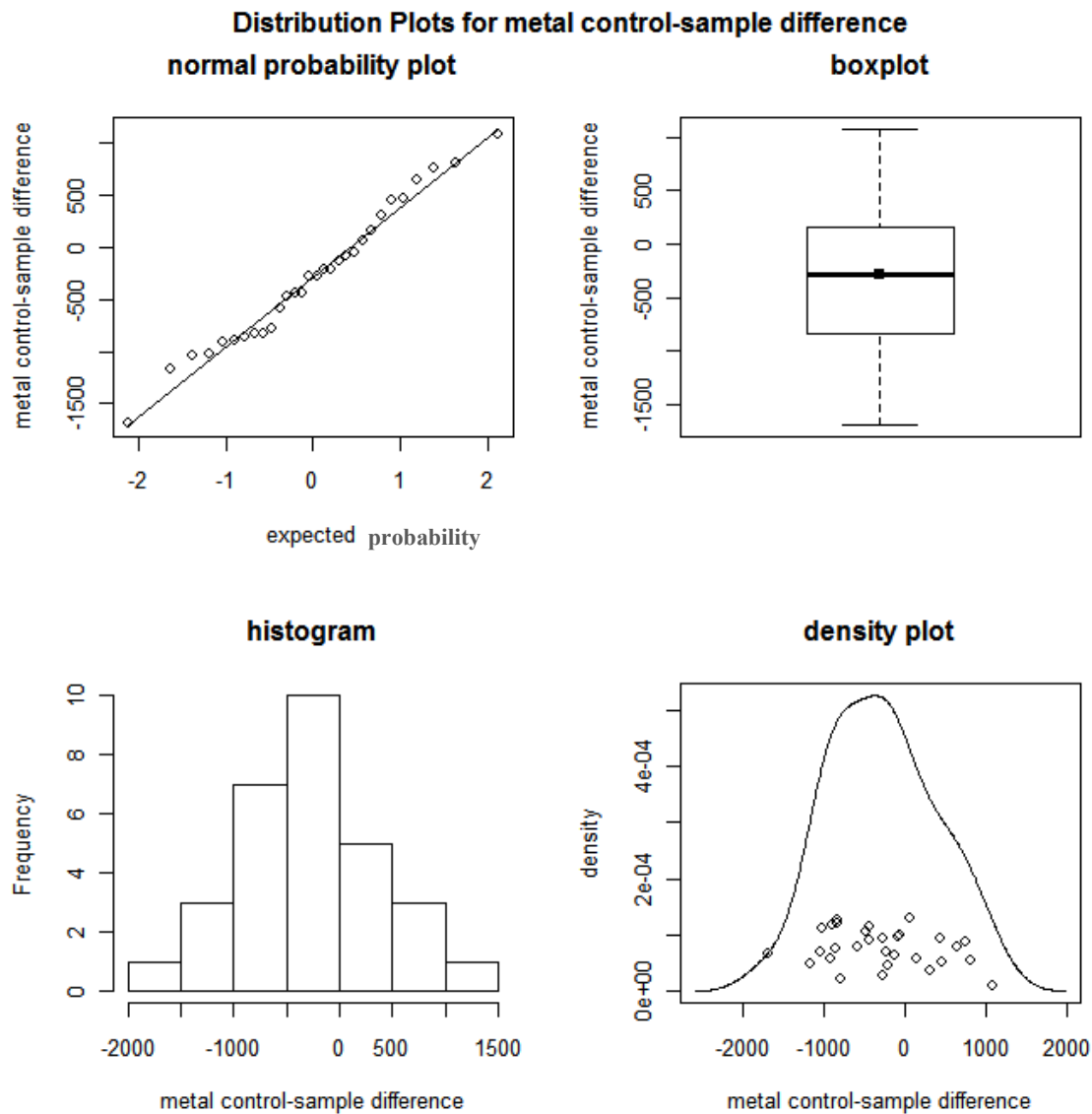


Figure 3. Distribution plots for the Sample-Control difference for samples 1 and 2 for metal buffer.

For metal control-sample difference, the normal probability plot (Figure 3) shows a good approximation to normality as was the case for the percent inhibition measure for the metal buffer. The remaining plots also confirm this normality property. Note that in the box plot, the histogram, and the density plot, the data are centered slightly below zero indicating slight bias.

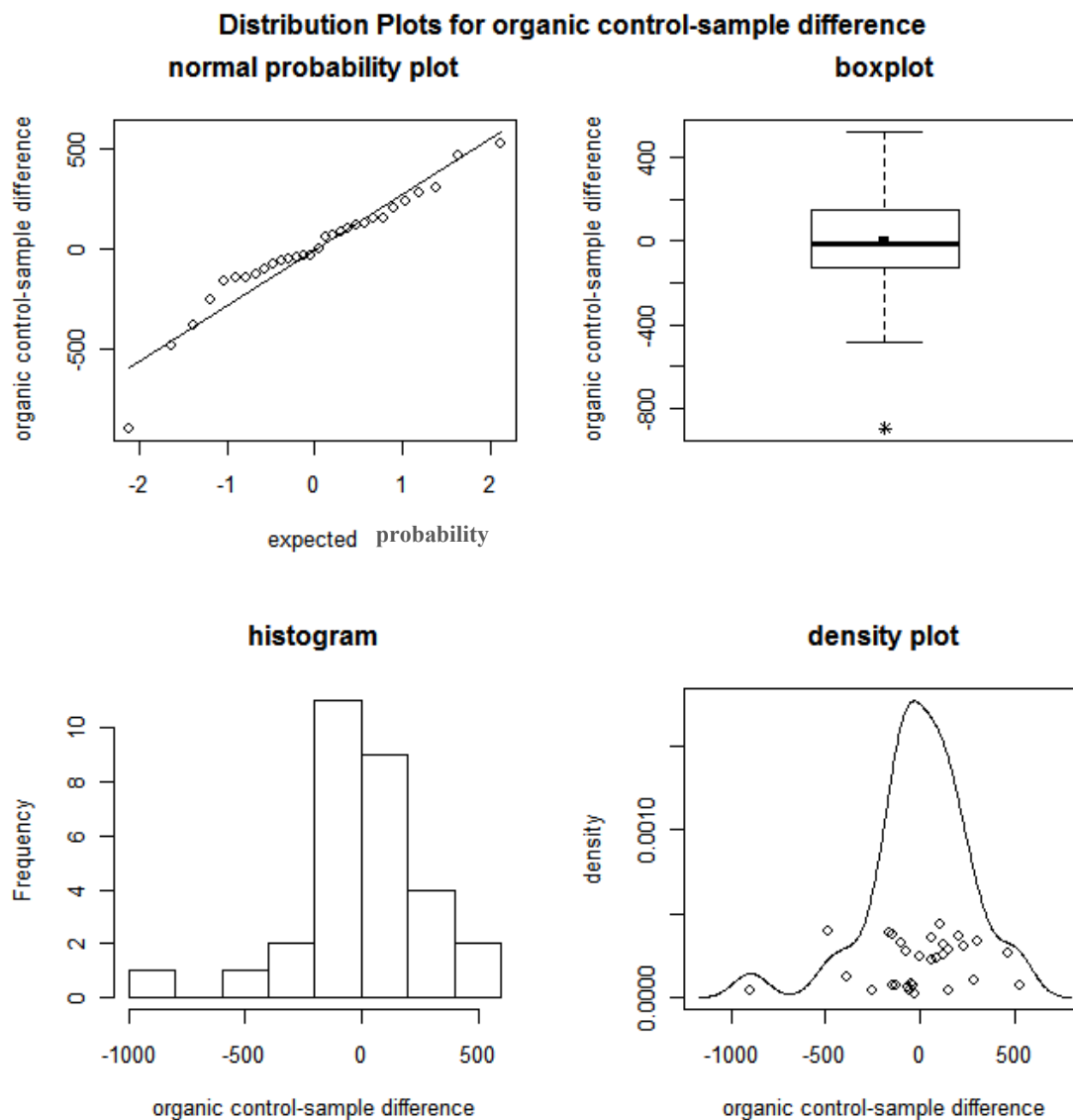


Figure 4. Distribution plots for the Sample-Control difference for samples 1 and 2 for organic buffer.

For the organic buffer control-sample difference, the normal probability plot (Figure 4) shows the data align fairly well along the diagonal line with the exception of one negative outlier. This outlier introduces an asymmetry of the remaining plots making the data appear skewed to the left. Other than this one point, the box plot, the histogram, and the density plot appear to be centered on zero as would be expected for a control-control assessment. This outlier occurs in sample 2 of test 13 and has a control-sample difference of -896.

Association of Difference to Mean

For the luminescence response, there appears to be no relation between the variability (difference between sample 1 and sample 2) and the mean (mean of sample 1 and sample 2) (Figure 5).

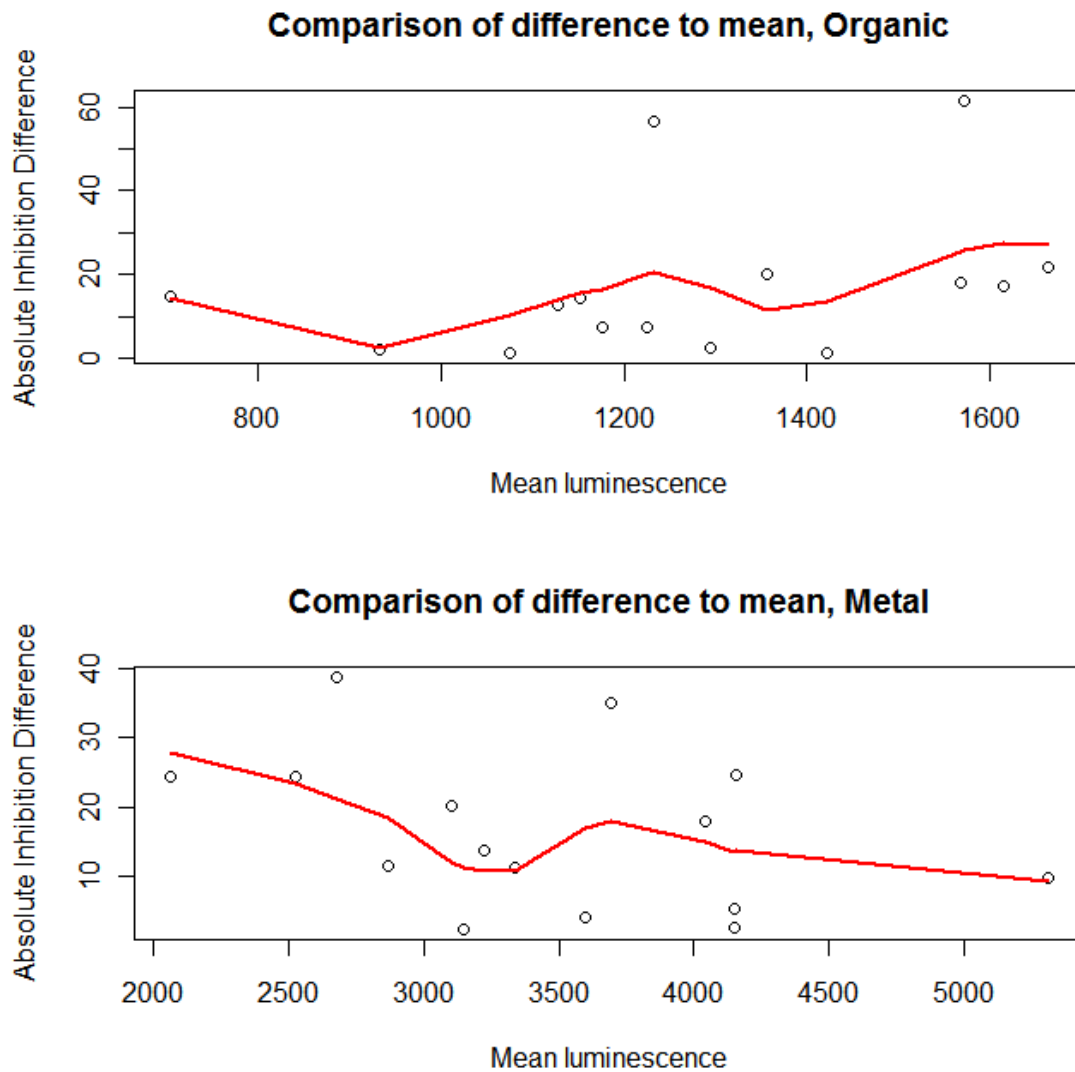


Figure 5. Comparison of absolute difference between samples to mean luminescence over samples for organic and metal buffers.

Tests for Bias, Normality, and Auto-correlation

For both the percent inhibition measure and the control-sample difference, the results for the metal buffer show a slight negative bias (Table 2; t-test p-value < 0.05, and Wilcoxon p-value < 0.05). The variability of these metal buffer measures is approximately normal (Shapiro-Wilk test > 0.05) and shows a little tendency toward autocorrelation (Durbin-Watson approximately = 0.05). For both the percent inhibition measure and the Control-Sample Difference, the results for the organic buffer show no evidence of bias (Table 2. t-test p-value > 0.05 and Wilcoxon p-value > 0.05) or autocorrelation (Durbin-Watson > 0.05). These measures do show some evidence of non-normality (Shapiro-Wilk test < 0.05), but this is due to a single outlier point.

Table 2. Summary of mean differences by buffer and variable

Buffer	Variable	Mean	standard deviation	t-test p-value	Wilcoxon test p-value	Shapiro-Wilk p-value	Durbin-Watson p-value
Metal	Pct Inhibition	-10.68	23.5	0.0109	0.0128	0.9444	0.0562
Metal	Control-Sample Difference	-283.93	664.63	0.0259	0.0308	0.8125	0.0615
Organic	Pct Inhibition	-2.32	21.68	0.4079	0.6408	0.0189	0.2863
Organic	Control-Sample Difference	-3.37	277.35	0.9319	0.7611	0.0661	0.212

Toxicity Threshold and False Positive Rates

Using the observed variability for the percent inhibition for the two buffers, the toxicity thresholds associated with fixed rates of false positive results are computed (Table 3). The false positive rate for 50% inhibition threshold for metal is 0.02475 or close to 5 in 200. The false positive rate for 50% inhibition threshold for organic is 0.00987 or close to 1 in 100. A false positive rate of 1 in 1000 corresponds roughly to a threshold of 79 for the metal buffer and 67 for the organic buffer if the test is considered two-tailed. A false positive rate of 1 in 1000 corresponds roughly to a threshold of 73 for the metal buffer and 62 for the organic buffer if the test is considered one-tailed.

Table 3. Toxicity thresholds for given false positive rate for percent inhibition.

Buffer	false positive rate (two tail)	false positive rate (one tail)	upper bound toxicity threshold
metal	0.05	0.025	41.87
	0.01	0.005	59.22
	0.005	0.0025	65.57
	0.002	0.001	73.26
	0.001	0.0005	78.66
	0.0001	0.00005	94.67
	0.00001	0.000005	108.56
organic	0.05	0.025	35.28
	0.01	0.005	49.89
	0.005	0.0025	55.24
	0.002	0.001	61.73
	0.001	0.0005	66.27
	0.0001	0.00005	79.76
	0.00001	0.000005	91.47

Discussion

The fact that there is strong test-to-test dependence (Table 1) shows that it is important to implement a negative control for each test to be used in standardizing the individual test measures. In this analysis two types of standardization were examined. One was a simple difference score where sample luminescence is subtracted from the negative control so that a reduction in luminescence is a positive score. The second standardization is this same difference divided by the negative control reading and scaled by a factor of 100 to yield percent inhibition. Both methods of standardization produce similar results in terms of the distributions being approximately normal (Figures 1-4, Table 2). Because the percent inhibition measure is on a 0-100 scale and is easier to interpret, it is preferred. Even after standardization, there appears to be some correlation between samples 1 and 2 in a test.

The variability of the response seems fairly independent of the mean response to the range of responses observed in this study (Figure 5). This result reinforces the conclusion that the normal distribution is a reasonable model for the variability of these data.

There is some evidence that both the difference response and percent inhibition have a negative bias for the metal buffer (Table 2). This evidence is not strong and perhaps it is just a sampling inconsistency in this experiment. If this trend is due to a real effect in that the sample readings tend to be higher than the negative control, then it will make the test prone to false negative results, which would be a serious error if toxicity is overlooked. The organic buffer did not exhibit this trend, which may make the data questionable for the metal buffer.

The organic buffer data do not conform to normality due to one extreme observation (test 13, sample 2). The fact that 1 out of 30 observations had percent inhibition exceeding 50% gives a false positive rate of 1 in 30, or 33 in 1000 for just this sample. For both the metal and the organic buffers, the false positive rate exceeds 1 in 100 for response threshold of 50%. If the response is viewed as a one-tailed indicator, the response threshold should be raised to 75% to obtain a false positive protection rate of 1 in 1000.

The data used for this report are as follows:

Raw Data from TOX-SPOT control-control test								
Buffer	ID	Negative Control	Sample 1 luminescence	Sample 2 luminescence	Sample 1 difference	Sample 2 difference	Sample 1 %Inhibition	Sample 2 %Inhibition
M	1	4039	4315	4096	-276	-57	-6.83	-1.41
M	2	5341	5033	5557	308	-216	5.77	-4.04
M	3	4105	4381	3642	-276	463	-6.72	11.28
M	4	3800	4381	4278	-581	-478	-15.29	-12.58
M	5	2801	2733	2047	68	754	2.43	26.92
M	6	2803	3240	3629	-437	-826	-15.59	-29.47
M	7	2768	3550	2987	-782	-219	-28.25	-7.91
M	8	3311	4174	4992	-863	-1681	-26.06	-50.77
M	9	2171	3089	3341	-918	-1170	-42.28	-53.89
M	10	3398	3243	4436	155	-1038	4.56	-30.55
M	11	2574	3470	3405	-896	-831	-34.81	-32.28
M	12	3163	3604	3243	-441	-80	-13.94	-2.53
M	13	4079	3276	3443	803	636	19.69	15.59
M	14	2566	1492	2121	1074	445	41.86	17.34
M	15	2291	2425	3315	-134	-1024	-5.85	-44.7
O	1	1179	1026	1176	153	3	12.98	0.25
O	2	1232	1268	1173	-36	59	-2.92	4.79
O	3	1050	1095	1081	-45	-31	-4.29	-2.95
O	4	1033	1288	1137	-255	-104	-24.69	-10.07
O	5	1498	1395	1371	103	127	6.88	8.48
O	6	625	697	789	-72	-164	-11.52	-26.24
O	7	1418	1470	1183	-52	235	-3.67	16.57
O	8	1857	1332	1653	525	204	28.27	10.99
O	9	1123	969	1606	154	-483	13.71	-43.01
O	10	1841	1372	1776	469	65	25.48	3.53
O	11	1363	1278	1240	85	123	6.24	9.02
O	12	1109	1252	1170	-143	-61	-12.89	-5.5
O	13	1225	1369	2121	-144	-896	-11.76	-73.14
O	14	1398	1527	1781	-129	-383	-9.23	-27.4
O	15	1131	847	823	284	308	25.11	27.23

Appendix C

Chemicals Evaluated

Appendix C: Chemicals Evaluated

Compound [measured analyte]	Chemical Abstracts Service Number ^a	Storage Requirements	Analytical Method	Source	Stability in Deionized Water	Purity %
Acrylonitrile [acrylonitrile]	107-13-1	4° C / dark	HPLC	Chem Service West Chester, PA	<3 hrs - open container; 14 days - no head-space vial	99.5
Aldicarb [aldicarb]	116-06-3	4° C / dark	HPLC	Chem Service West Chester, PA	>14 days	99
Ammonium chloride [total ammonia]	12125-02-9	4° C / dark	colorimetric	Sigma-Aldrich St. Louis, MO	>14 days	99.99
Sodium arsenite [As]	7784-46-5	4° C / dark	ICP-MS	Chem Service West Chester, PA	>14 days	98
Sodium azide [azide]	26628-22-8	4° C / dark	Ion Chromatograph	Sigma-Aldrich	>14 days	99.5
Chloramine [monochloramine]	10599-90-3	4° C / dark	amperometric titration	Sigma-Aldrich	24 hrs	NA
Sodium hypochlorite [chlorine residual]	76881-52-9	4° C / dark	amperometric titration	Riedel-de Haën Fine Chemicals Seelze Germany	>14 days	NA
Copper sulfate [Cu]	7758-99-8	4° C / dark	ICP-MS	Sigma-Aldrich	>14 days	99.95
Sodium cyanide [cyanide]	143-33-9	4° C / dark	ion probe	Sigma-Aldrich	>14 days	99.98
Fenamiphos [fenamiphos]	22224-92-6	room temp / dark	Nominal	Chem Service	>14 days	98.5
Sodium fluoroacetate [fluoroacetate]	62-74-8	4° C / dark	HPLC	Sigma-Aldrich	> 14 days	>90
Geosmin	19700-21-1	4° C / dark	Nominal	Sigma-Aldrich	not measured ^b	98
Humic/fulvic acid mixture (1:1 by weight)	NA	4° C / dark	Nominal	International Humic Substances Society, St. Paul, MN	not measured ^b	NA
Mercuric chloride [Hg]	7487-94-7	room temp / dark	ICP-MS	Sigma-Aldrich	>14 days	99.5
Methamidophos [methamidophos]	10265-92-6	4° C / dark	Nominal	Chem Service West Chester, PA	>14 days	98.8
Methyl parathion [methyl parathion]	298-00-0	4° C / dark	HPLC	Chem Service West Chester, PA	>14 days	99.3
2-methylisoborneol (MIB)	2371-42-8	4° C / dark	Nominal	Sigma-Aldrich	not measured ^b	98
Nicotine [nicotine]	54-11-5	4° C / dark	HPLC	Chem Service West Chester, PA	>14 days	99.4
Paraquat dichloride [paraquat]	1910-42-5	4° C / dark	HPLC	Chem Service	>14 days	99
Sodium pentachlorophenate [pentachlorophenate]	131-52-2	4° C / dark	HPLC	Mallinckrodt Baker Phillipsburg, NJ	>14 days	99
Phenol [phenol]	108-95-2	4° C / dark	HPLC	Sigma-Aldrich	>14 days	99.5
Thallium sulfate [TI]	7446-18-6	4° C / dark	ICP-MS	Sigma-Aldrich	> 14 days	99.995
Toluene [toluene]	108-88-3	4° C / dark	HP6890 GC and HP-7694 HS	Sigma-Aldrich	14 days; no-head space vial	99.8

GC = gas chromatography LC-MS = liquid chromatography – mass spectrophotometry ICP-MS = inductively coupled plasma-mass spectrophotometry
HPLC = high performance liquid chromatography HP6890 GC and HP-7694 HS = gas chromatography & head-space sampling NA = Not available

^a Number for compound ^b Tested within 24 hrs of preparation