Water Toxicity Testing
Applications Guide

Version 1.0

1. Introduction- Technology Overview

1.1 Why is it important to determine chemical contamination in water?

The quality of potable water is one of the most important elements for human health. Intense development of the chemical industry, and the use of pesticides in agriculture can result in contamination of natural water resources. Moreover, chlorinating of water (used to inhibit bacterial contamination) may lead to the generation of complex mixtures of toxic and genotoxic chlorinated hydrocarbons in drinking water. In the absence of a rapid, sensitive, general, and cost-effective test for such a diverse group of contaminants, however, most water supplies are not monitored on a frequent routine basis.

1.2 The principle of the TOX-SCREEN test

The use of intact luminous bacteria for toxicity assessment has some clear advantages that have been scientifically validated (Bulich and Isenberg,1981; Kaiser,1998; Ulitzur et al, 2002): luminous bacteria are self-maintained luminescent units that, under proper conditions, emit high and steady levels of luminescence (490nm). The light of several hundred cells may be determined with a simple luminometer. Chemo-physical and biological toxicants that affect cell respiration, electron transport systems, ATP generation, the rate of protein or lipid synthesis, alter the level of luminescence. Similarly, agents that affect the cell's integrity and especially membrane function, have a strong effect on in vivo luminescence. Hence, toxicants of different characteristics such as, pesticides, herbicides, chlorinated hydrocarbons, heavy metals etc, exert a dramatic and measurable effect on the bacterial luminescence system. By comparing the luminescence level obtained in a suspected toxic sample with that obtained in a clean water-control sample after a short period of incubation, one can detect very low concentrations of a broad range of toxicants.

The TOX-SCREEN test is based on using luminous bacteria as very sensitive and accurate biosensors. The operation principle is simple – changes in the level of bio-luminescence indicate potential toxicity. The test kit includes Assay buffers, freeze-dried luminous bacteria, positive control solutions, and plastic tubes. A luminometer is required to read the results. The testing procedure is simple as wel; only 1mL of sample is necessary to run the assay, making collection, storage, and disposal of sample material easy and non- expensive. Low cost per test encourage frequent testing for rapid response to changing conditions in water quality. The tests can be performed **either in the lab or on site** and can be used by water companies, health and environmental supervising authorities, municipal water system authorities, hospitals, military units, private well owners, lake, stream and river monitoring agencies etc.

1.3 Why are is the TOX-SCREEN technology more sensitive than other short- term bioluminescence tests?

The higher sensitivity to diverse groups of toxicants with comparison to other bioluminescence-based tests is due to the selection of a highly sensitive variant of *Pothobacterium leiognathi,* an improvement of its lyophilization procedure and the selection of special assay conditions. This unique combination resulted in 10 to 100 folds increase in sensitivity for most of the important toxic agents. Two assay buffers were developed, one which favors the detection of cationic heavy metals and metalloids (Pro-Metal Buffer),

and the other favors the detection of organic contaminants (Pro- Organic Buffer). These buffers were developed to enhance the sensitivity of the test to a wide range of toxic agents with different modes of action. When tested concurrently, the buffers are generally able to discriminate between the presence of organic and metal agents at sub mg/L concentrations.

Using TOX-SCREEN in the lab

1.4 What is unique about the TOX-SCREEN^3 Test and how is it different from the TOX-SCREEN-I and -II Test?

The first versions of the test (ToxScreen-I, ToxScreen-II) were verified by the USEPA-ETV in 2003, and 2006, respectively). The modified **TOX-SCREEN^3** test (introduced in late 2007) offers increased sensitivity for many chemical contaminants, rapid results (reaction time shortened from 60 minutes to 15 minutes), added flexibility due to reagent work week-long utilization. The test is based on the effect of toxic agents on the development of luminescence in the natural marine bacterium *Photobacterium leiognathi* (strain SB) that is more temperature-tolerant than the *Photobacterium leiognathi* variant TANI-1 used in the TOX-SCREEN-I version.

Sensitivity - the TOX-SCREEN^3 test exhibits improved sensitivity to a wide range of toxic agents, some of which are known as potential terrorism hazards (arsenic, cyanide) and others (2,4-D, 2,4,5-T, and chromium). Unlike *Vibrio fischeri* or even the TANI-1 variant, the bacterial strain used is very tolerant to a wide range of temperatures (18°-35°C), though the optimal temperature for rapid response is 30°C.

Discriminatory – a unique assay buffer set (Pro-Metal & Pro-Organic) enables preliminary discrimination between organic toxicants and cationic heavy metals(& metalloids).
Flexible- different testing protocols can be used (quantitative/qualitative).

Cost efficiency - an important advantage of the new test is the extended usability of the suspended reagent; unlike the TOX-SCREEN-I test version, the hydrated luminous bacteria are transferred into Storage Buffer and stored at 2°-4°C. Around 100 single tests may be drawn from this suspension for up to 5 days. During the 5 days storage, light output diminishes but sensitivity and reliability of results remains stable. The bacterial suspension is added to the tested water to which a buffer, containing certain salts and metabolites, is added by the user. In the absence of toxicants the in vivo luminescence remains quite stable at 30°C. For most of the toxic agents tested, the new assay was markedly more sensitive than comparative bacterial bioluminescence toxicity data reported in the literature with other bioluminescence-based short-term tests.

1.5 TOX-SPOT test – Specially Designed for On-Site Testing

A new version of the technology has been introduced in early 2010 that is specially designed for on-site testing. While the TOX-SCREEN test kit is designed for **routine, lab testing**, for screening large number of samples and/or determination of relative toxicity. TOX-SPOT is designed for **on-site, field testing** under emergency situations or occasional screening.

The test exhibits similar sensitivity as the TOX-SCREEN[3] test and has the following features:

On site real time results - one of the key features of the new test is its rapid response. Optimal results can be obtained on site within 15 minutes. The kinetics of response largely depends on the nature of toxic chemical and its concentration. Hence, in many cases a detectable effect on luminescence will be observed within a few minutes of exposure. When real-time results are crucial, one could record luminescence after 5 and 10 minutes.
User friendly & robust- minimal skill and training is necessary. Can be used anywhere.
Cost effective- the robustness of the SB *P. leiognathi* variant enables on-site testing with low cost photodiode-based luminometers. In addition, the stability of the freeze-dried bacterial reagent preparation at ambient temperatures precludes the need for refrigeration or freezing during shipment contributes to further reducing overall operational costs.

TOX-SPOT Refill Kit

TOX-SPOT Starter Kit

2.1 Choosing The Right Test Protocol – Drinking Water Applications

Application		Tested Water Source	Frequency of Testing		Reference Water Control Source[1]	Dilute Sample Before Testing	Requires Baseline Setting[2]	Recommended Test Protocol[2]	
			Routine	Emergency				Qualitative (Go-No Go)	Semi-quantitative (degree of toxicity)
Drinking water	Raw	Low to mild pollution: River, lake, stream, well	+		Clean river; local mineral	NO	YES	Protocol 3	Protocol 1
				+	Clean river; local mineral	NO	NO	Protocol 2 or SPOT	–
		Mild to heavy pollution: River, lake, stream, well	+		Double distilled	YES	YES	Protocol 3	Protocol 1
				+	Double distilled	YES[3]	NO	Protocol 2 or SPOT	–
	Treated/ Finished	Reservoir, tank,pipe, tap	+		Local mineral	NO	YES	Protocol 3	Protocol 1
				+	Local mineral	NO	NO	Protocol 2 or SPOT	–

1 - if none of these options is possible, use double-distilled water
2 - Consult TOX-SCREEN user guide for details
3 – Run preliminary testing before emergency event to determine degree of dilution using Protocol 1

2.2 Choosing The Right Test Protocol – Environmental Monitoring Applications

Application	Tested Water Source	Frequency of Testing		Reference Water Control Source[1]	Dilute Sample Before Testing	Requires Baseline Setting[2]	Recommended Test Protocol[2]	
		Routine	Emergency				Qualitative (Go-No Go)	Semi-quantitative (degree of toxicity)
Environmental monitoring	Low to mild pollution: River, lake, stream, well	+		Clean river; local mineral	NO	YES	Protocol 3	Protocol 1
			+	Clean river; local mineral	NO	NO	Protocol 2 or SPOT	–
	Mild to heavy pollution: River, lake, stream, well	+		Double distilled water	YES	YES	Protocol 3	Protocol 1
			+	Double distilled water	YES[3]	NO	Protocol 2 or SPOT	–

1 - if none of these options is possible, use double-distilled water
2 - Consult TOX-SCREEN user guide for details
3 – Run preliminary testing before emergency event to determine degree of dilution using Protocol 1

2.3 Repeat Dispensing in the TOX-SCREEN Test- Recommended Products

All of CheckLight's **lab test procedures** include the dispensing of very low volumes (10 microliters) of hydrated bacterial suspension into the assay mix.
Since the overall time span of each test is short and light emitted by the bacteria in the sample is compared to light emitted in the negative control, one has to ensure rapid dispensing. Moreover, as each 10 microliter aliquot holds about one million cells, dispensing 9 or 11 microliter, leads to a dramatic change in emitted light, and hence, to skewed results. It is therefore essential to ensure accurate dispensing.

Given the above, the use of an automatic pipettor or repeat dispenser provides the optimal solution and should be regarded as an essential tool.

There are numerous products on the market. Among the recommended options for highly reliable products are:

1. Finnpipette Stepper model 4540 from Thermo Scientific-
http://www.thermo.com/com/cda/product/detail/0,1055,19353,00.html

2. Ripette electronic pippetor from Ritter -
http://www.ritter-online.de/e/medical_care/ripette/index.php

A less sophisticated line of products is the syringe-less version.

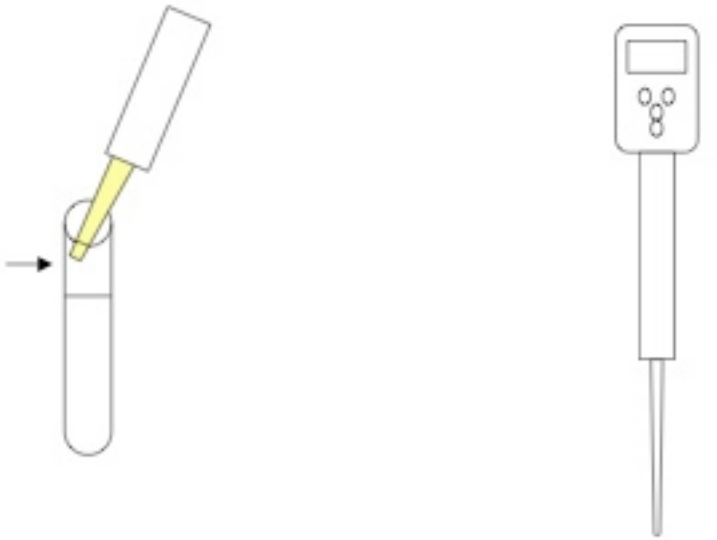
We provide a product manufactured by Microlit -
http://www.microlit.com/elec.htm

Instructions for use are provided in the next page.

A short training video clip on pipetting is also avaliable for download on our web site.

2.3.1 Instructions For Operating The Electronic Micropipette (Repeat Dispenser) With TOX-SCREEN Test Kit

1. The device has 3 operation modes to choose from. The one relevant for use with CheckLight's kits is **CASE III – Stepper Mode**.
2. Follow the instructions for setting the Stepper Mode in the provided Operation Manual.
3. During the dispensing phase it is very important to **touch the inner side of the tube with the edge of the dispensing tip** in order to ensure that each drop is released and captured in the tube.



2.4 Testing the Toxicity of Chemicals With Low Water Solubility Using TOX-SCREEN^3

2.4.1 Introduction

When running eco-toxicological bioassays, the use of organic solvents in unavoidable since many organic pollutants (especially pesticides) have low water solubility and need to be dissolved in organic solvents prior to addition into experimental systems.
The USEPA recommends maximum allowable limits of 0.05% solvent for acute tests (Jay 1996). But in professional publications, the nature of the solvent and the final concentration used vary among different authors and are often higher than USEPA limits due to problems associated with the use of small test volumes and toxicant solubility (Jay 1996). Organic solvents can cause toxic effects on their own, but it has been reported that they can interact with pesticides and alter toxicity. To ensure that bioassay data are accurate and not the result of solvent interference, a screening method is available to identify and minimize solvent interactions in such bioassays (Stratton, 1985, 1989; Stratton and Corke, 1981; Stratton and Smith, 1988; Stratton et al, 1982). First, choosing a solvent for use in bioassays should involve a detailed screening to identify solvents with inherently low toxicity to the test organism, followed by an interaction study involving pesticide and solvent interactions to choose the best concentrations to use (Stratton and Smith, 1988).

2.4.2 How to run spiking tests of low-solubility chemicals with TOX-SCREEN^3

2.4.2.1 Sensitivity to common solvents

There are numerous solvents that could be safely used in the TOX-SCREEN^3 bioassay – ethanol, acetone, and DMSO. Their potential toxicity was tested and found to be as follows –

Solvent	**IC50** (The concentration that leads to 50% inhibition in emitted light after 15 minutes incubation at 30°C)
Ethanol	1%
Acetone	2%
DMSO	5%

2.4.2.2 Preparing stock solutions of chemicals

A stock solution of a chemical with low solubility in water should be prepared in the solvent recommended by the manufacturer. The concentration of the chemical in the stock solution should be calculated so that once diluted in water the final solvent concentration in the test would not exceed the IC50 of the solvent.

For example, if one wants to test 10ppm (mg/L) of Malathion, then a stock solution of no less than 500 ppm should be prepared in acetone, so that once diluted x50, the final concentration of acetone would not exceed 2% (100:50=2).

2.4.2.3 Test set up and controls

Next, the stock solution is diluted in clean water to reach the desired concentration to be tested. This could be done either by a step dilution, or serial dilution. The final concentration of the solvent should be calculated and a negative control (reference) sample containing that solvent concentration should be prepared.

Using the above example, a stock solution of 500 ppm Malathion is prepared in acetone, diluted 1:50 in clean water to a final concentration of 10 ppm. The negative control for such test would be 2% acetone in clean water.

3. Data Analysis Guidelines

TOX-SCREEN and TOX-SPOT are **broad-based screening** technology designed to detect changes in toxicity. It is a vital part of the First Tier of an Early Warning System. A positive result from the first stage would trigger the second stage (Second Tier) of **confirmatory analysis** using more **specific and sensitive techniques**. A positive result from the confirmatory analysis would trigger a **response action**. The alarm should be triggered by a **combination of events**, not a single detection, which may be a false positive.

Utilizing the tests for measuring water toxicity generates data from two parallel assay systems (one favoring the detection of cationic heavy metals; the other favoring the detection of organic toxicants) and inhibition concentrations ranging from 0 % to 100% normalized against the clean reference control.

In order to simplify data interpretation and report output, please consult the table below. In cases where mildly to heavily contamination is noted, it is highly recommended to repeat the test.

Case #	Light Inhibition in Pro-Organic Buffer	Light Inhibition in Pro-Metal Buffer	Report Output
1	>75%	>75%	Sample heavily contaminated [>75%]
2	>75%	41-74%	Sample heavily contaminated [>75%]
3	>75%	<40%	Sample heavily contaminated [>75%]
4	41-74%	>75%	Sample heavily contaminated [>75%]
5	<40%	>75%	Sample heavily contaminated [>75%]
6	41-74%	41-74%	Sample mildly contaminated [41-75%]
7	<40%	41-74%	Sample mildly contaminated [41-75%]
8	41-74%	<40%	Sample mildly contaminated [41-75%]
9	<40%	<40%	Sample not contaminated [<40%]

4. Using The Kit as Part of an Early Warning System for Determining Dangerous Changes in Drinking Water Quality

4.1 Introduction

This test is meant to act as a routine daily screen of a drinking water source. If the result exceeds the threshold the samples should be forwarded to chemical analysis to determine the exact nature of the contamination.

Step 1

Collect water samples throughout the distribution network (for example source, before treatment, after treatment, reservoirs, tanks, taps) and test them according to Protocol 3 (TOX-SCREEN user guide). It is recommended to test duplicates of each sample to reduce errors. Since toxicity of a water source may vary with season, temperature and time of day, take special care to carefully mark these variables in your log book.

For each water sample, determine:

Question - Is light inhibition greater than 50%?
Answer 1 - YES – moderate to high level of toxicity - proceed to Step 2.
Answer 2 – NO – low to no detectable toxicity –proceed to Step 3

Step 2
Re-test the samples using Protocol 1 to determine the degree of toxicity (i.e., how much can the sample be diluted and still exhibit 50% inhibition?)

Step 3
Keep using Protocol 2 for routine measurements.

Day to day operation
It is very important to establish a base line reading profile for each sampling point. This will enable the determination of a toxic event for each point once it occurs. Toward that end you will need to calculate the average reading of at least 15 data points from each sampling source. Next, determine the standard deviation (preferably 3xSD) to set the threshold level beyond which dangerous change in water quality is noteworthy. In cases where toxicity threshold is exceeded, sample should be forwarded to chemical analysis in order to try and determine exact toxicity cause and nature.

4.2 Example of Screening Results

Date	Tested tube	Luminescence (RLU)	Inhibitory Concentration (%)
12.1.07	Sample	18562	17.5%
	Control	22500	0
13.1.07	Sample	16560	34.8
	Control	25400	0
14.1.07	Sample	16681	21
	Control	21009	0
15.1.07	Sample	21441	13.7
	Control	24845	0
17.1.07	Sample	21745	20
	Control	27182	0
18.1.07	Sample	20732	18.5
	Control	25439	0
19.1.07	Sample	17399	17.5
	Control	21090	0
20.1.07	Sample	18058	14
	Control	20998	0
23.1.07	Sample	18514	9
	Control	20346	0
24.1.07	Sample	18431	11.8
	Control	20897	0
25.1.07	Sample	13168	30.7
	Control	19002	0
26.1.07	Sample	12941	34.9
	Control	19879	0
27.1.07	Sample	13629	24.6
	Control	18076	0
28.1.07	Sample	16870	10.9
	Control	18934	0
2.2.07	Sample	15717	13.7
	Control	18212	0
28.1.07	Sample	11382	36.8
	Control	18010	0

Note- the shown values are for illustration only

Average	**20.56**
Standard Deviation	**9.14**
Standard Deviation (x3)	**27.42**

The IC (Inhibitory Concentration) is calculated as: 100 x [1 - (RLUsample/RLUcontrol)] and is expressed as percent (%). In this example, the threshold level is set at 27.42.

Step 2 – Routine Screening
Sample ID - 001 ; Assay Buffer – Pro-Organic

Testing Date	Test	RLU	Relative Activity (%)	IC (%)
1.3.07	Sample Control	6500 8240	78.8 100	21.2 0
3.3.07	Sample Control	6100 8122	75.1 100	24.9 0
5.3.07	Sample Control	6813 8030	84.8 100	15.2 0
7.3.07	Sample Control	6771 8001	84.6 100	15.4 0
9.3.07	Sample Control	4930 7993	61.6 100	**38.3** 0

Data Analysis
The sample tested on 9.3.07 exceeded the predetermined threshold level (IC=27.42%). It should be retested and if confirmed should be considered as suspected to contain a concentration of toxic chemical(s) above normal for that specific water source.

Recommended reading: Water Security Initiative: Interim Guidance on Planning for Contamination Warning System Deployment (Office of Water EPA 817-R-07-002 May 2007).

5. Using The Kit For Effluent or Heavily Polluted River/Lake Toxicity Monitoring

5.1 Introduction

Sewage treatment plants are based on the use of bacteria to breakdown the sewage. The bacterial may be harmed by toxic substances that enter the sewage plant. The kit is an ideal solution for monitoring the inflow of sewage treatment plants to avoid possible inhibition of microbial processes in the activated sludge due to toxic discharges.
Since each sewage plant has a different profile of inflow toxicity, the plant-specific threshold level must be determined (described below). Due to the high sensitivity of the test and the concentrated nature of sewage, it is usually necessary to dilute the tested water by up to a few hundred folds before determining luminescence inhibition profile. This dilution minimizes possible interference due to extreme pH, presence of suspended particles, colorants, etc.
The same logic applies to testing heavily polluted river/lake toxicity monitoring.

5.2 Procedure

1. Collect a few inflow samples and prepare a set of serial dilutions from each sample (e.g., from 10% down to 0.01%)
2. Determine the dilution level that resulted in about 20% inhibition in luminescence (IC20).
3. For the next 2-3 weeks of normal plant operation, test the above-determined dilution and record degree of inhibition.
4. Determine the threshold level from the collected data by finding the highest inhibition level at which the plant functions properly or according to the following equation:

   Threshold level (in %) = Average value + (Standard Deviation) x 3

   Or, choose the highest inhibition level at which the plant functions properly.

5. Once threshold level is determined, continue monitoring on a routine basis. Luminescence inhibition levels that exceed the threshold may mean that there is a dangerous toxic inflow that must be diluted before reaching the activation stage.
6. In addition, it is recommended to test the toxicity of the undiluted outflow for reliable monitoring of sewage treatment efficiency.

Note: steps 1 and 2 need only be determined once for each water source.

Step 1 – what dilution of sample exhibits 20% inhibition in luminescence?

Date -12.12.00 Assay Buffer -Pro-Metal/Pro-Organic Sample ID -001 Time/Temp. -10min/30°C

Vial No.	Sample Conc. (%)	Luminescence (RLU)	Relative Activity (%)
1	10	350	4.1
2	5	1006	12.02
3	2.5	2450	29.2
4	1.25	5012	59.9
5	**0.62**	**6690**	**80**
6	0.31	6820	81.5
7	0.156	6905	82.5
8	0.078	7200	86
9	0	8750	100*
10	0	7980	

Note- the shown values are for illustration only

5.3 Calculations:

100% activity is the reading when there are no toxic compounds present that may reduce luminescence. This figure is calculated by taking the average of the two negative controls (Vials 9 and 10); 8750 + 7980/2= 8365. Therefore 8365 is defined as 100% activity.

Relative Activity is calculated as: 100 x (RLU sample/RLU control)

For example, in the table above Relative Activity for vial no.1 is calculated as: 100 x (350/8365) = 4.1%, etc . In the example above - when the tested sample was diluted 161.3 fold (vial 5 - final concentration of 0.62%) it exhibited 80% Relative Activity, or, 20% inhibition.

This step only needs to be performed once. For example, in this sewage plant all future assays will be performed with the water sample diluted to 0.62%.

Step 2 – what is the threshold level of the sample?

Sample ID -001 Tested sample concentration – 0.62% Assay Buffer – Pro-Metal/Pro-Organic

Testing Date	Vial no.	RLU	Relative Activity (%)	IC(%)
1.1.01	1 - sample	6500	79.2	20.8
	2 – control	8206	100	0
3.1.01	3 - sample	6100	77.1	22.9
	4 – control	7910	100	0
5.1.01	5 - sample	6650	77.8	22.2
	6 – control	8540	100	0
7.1.01	7 - sample	6210	76.4	23.6
	8 – control	8120	100	0
9.1.01	9 - sample	6710	85	15
	10 - control	7890	100	0
Average				**20.9**
SD				**3.39**
Highest IC				**23.6**
Threshold (Mean + 3SD)				**31.08**

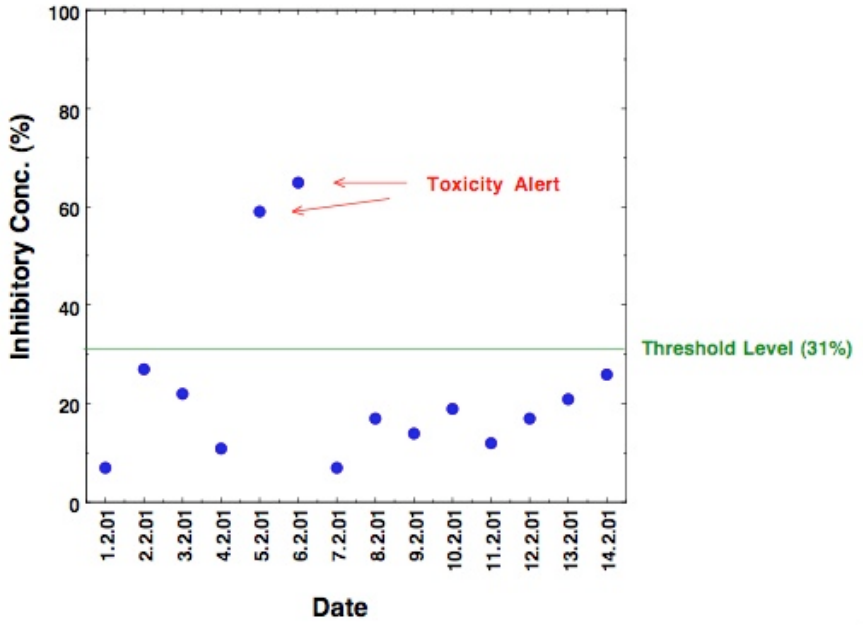
Note- the shown values are for illustration only

Calculations

The IC (Inhibitory Concentration) is calculated as: **100 x [1 - (RLUsample/RLUcontrol)].** Hence, the sample tested on 1.1.01 showed: 100 x [1-6500/8206)]= 20.8% inhibition. Etc.

Step 3 – routine monitoring for changes in toxicity level

Date -1-14.2.01 Tested concentration – 0.62% Sample ID -001 Assay Buffer – Pro-Metal/Pro-Organic



6. Potential Interferences

6.1 How To Handle Potential Interferences

Optimal Working Conditions		Corrective Measures
pH range	6.0 - 8.5	If the toxic effect of the pH is not wanted, adjust pH of the sample before testing with 0.5N HCl (to decrease pH) or 0.5N NaOH (to increase pH).
Turbidity (NTU)	0 - 50	If the toxic effect of turbidity is not wanted, highly turbid samples (>50NTU) need to be centrifuged (in the lab) or filtered (on site) before testing. Do not use PVDF, cellulose acetate or cellulose nitrate filters.
Color	None	Highly colored samples (black, red, brown) will absorb light and effect the results. If the toxic effect of the color is not wanted, sample should be diluted before testing.
Chlorine	<0.1 ppm	Up to 4 ppm residual chlorine can be neutralized with 2 ppm sodium thiosulfate. In TOX-SPOT there is an option to order assay buffers that already contain thiosulfate.

6.2 Guidelines For Handling Turbid Environmental Samples

Turbidity is a physical property of water that describes its clarity: "cloudy" waters are turbid. Turbidity is due to the scattering of light by small particles. These particles can be inorganic (e.g., silt and clay) or organic (e.g., algae). Samples drawn from surface water sources tend to contain variable concentrations of suspended particles. Beyond a certain concentration, these particles affect light scattering, and hence change the recorded level of light emitted by the luminescent bacteria used as biosensors in the toxicity test.

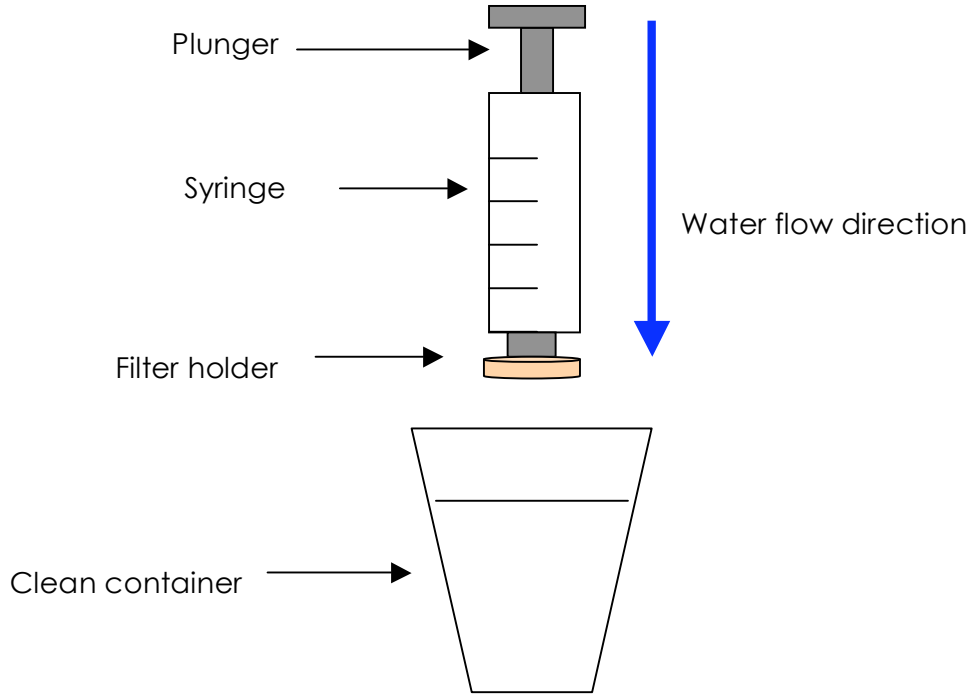
The most reliable method for determining turbidity is nephelometry (light scattering by suspended particles) which is measured by means of a turbidity meter giving Nephelometric Turbidity Units (NTU). As long as the turbidity level is less than 50 NTU, there is no danger that the particles will interfere with the test. Samples that are more turbid (>50 NTU), or contain particulate matter that will not settle, require clarification before testing.

It is important to remember that toxicity may be associated with the turbid components of the sample. It is well documented that many kinds of toxic substances often adhere to sediment particles. If the toxic effect of turbidity is not wanted, highly turbid samples (>50NTU) need to be centrifuged (in the lab) or filtered (on site) before testing. A brief description of on site filtration using a filter-syringe is provided below. Do not use cellulose acetate or cellulose nitrate filters.

If determining the toxic effect associated with the particles is important, run the test on the sample as is. In case of uncertainty about the nature of potential contamination (i.e., toxicity) in the sample, it is best to run a test on both filtered and non-filtered versions of the sample. As in any toxicity test, it is always important to use clean reference water (i.e., negative control) that is closest in nature (mineral content, pH, etc) to the tested sample.

6.2.1 Using a filter syringe to remove particles from a sample before testing:

1. Remove the plunger from the syringe and attach the filter holder to the bottom of the syringe.
2. Pour approximately 5 ml of sample water into the barrel of the syringe, replace the plunger into barrel and exert pressure on the plunger to slowly force the water through the filter. Collect the water in a suitably clean container.
3. Remove filter holder from syringe then remove plunger from bottle. This procedure is required to prevent rupturing the membrane filter by the vacuum created as the plunger is removed from the barrel.



- It is possible to prepare up to 100 ml of turbidity-free water using a single filter.
- Periodically examine the membrane filter to insure no holes or cracks are evident.
- The membrane filter may be stored in the holder for an indefinite period of time and used as required.

7. Frequently Asked Questions

Q: What is a toxicity test?
A: A toxicity test can be considered a bioassay that allows measurement of damage. It is a measure of the degree to which a substance can elicit a deleterious effect (including death) in a given organism.

Q: How can luminous bacteria sense water toxicity?
A: Luminous bacteria emit measurable light as a by-product of cell respiration. Chemo-physical and biological factors that affect cell respiration, promptly alter the level of luminescence. Similarly, factors that affect the cell's integrity, and especially membrane function, have a strong effect on in vivo luminescence. Hence, by simply comparing the luminescence level obtained in the suspected toxic sample with that obtained in the control (clean water sample), one may detect very low concentrations of a broad range of toxicants.

Q: What are the advantages of using a bioassay for environmental monitoring?
A: Bioassays employ biological systems to detect toxicants in environmental samples (e.g., effluents, water, sediments, or soil) under investigation. The primary advantage of using bioassays is that toxicity can be evaluated. The use of bioassays provides a holistic approach that allows the toxicity evaluation of the total integrated effect of all constituent components, including toxicants and confounding variables, in a given complex sample matrix. The net assessment is the combined interactive evaluation of additive, antagonistic and synergistic affects of all sample components.

Q: Can the test replace chemical analysis?
A: As a general rule, toxicity testing is never a substitute for chemical analysis. The test provides a rapid and sensitive tool for first response assessment of water contamination. An indication of a dangerous change in water quality should lead to a comprehensive analysis and/or emergency response.

Q: How is CheckLight's toxicity test different from other bioluminescence-based tests?
A: For most water toxicants tested, CheckLight's test was found to be many folds more sensitive than other bioluminescence-based tests. Unlike these tests, CheckLight's TOX-SCREEN & TOX-SPOT tests can be run at a wide temperature range (20°C-30°C). Moreover, a unique dual buffer set allows the discrimination between cationic heavy metals and organic toxicants.

Q: Are luminous bacteria dangerous? Do I need to be a trained microbiologist in order to be able to conduct CheckLight's assays?
A: Luminous bacteria are not pathogenic and are harmless. No special skill is required to carry out the different tests other than very basic laboratory techniques (pipetting, dilutions etc) and equipment (pipettor, tips, luminometer).

Q: Why is there a control in each assay?
A: Readings of the control are needed to calculate the relative luminescence inhibition by the sample toxicant. Fixing the reading from an unaffected control at 100% bioluminescence (0% toxicity) and reading the sample compared to it is the accepted method.

Q: How might chlorinated water affect luminescence?
A: Chlorine is usually introduced into drinking water systems in order to avoid bacterial contamination. Since luminous bacteria used in the assay are also sensitive to this treatment, one should add sodium thiosulfate to the assay to dechlorinate the sample before adding the bacteria. When the bactericidal effect of chlorine is in question, samples with or without sodium thiosulfate may be used to evaluate the bactericidal activity of chlorine under the studied conditions.

Q: What does the term EC50 mean and how do I calculate it?
A: The degree of water toxicity is expressed in relative values, termed EC50 or IC50, that is defined as the minimal effective concentration of the tested water (in %) that results in 50% inhibition of the light level obtained in the clean water control sample under defined assay conditions. The provided software assists you in automatically calculating this value from the generated data.

Q: Can I "play around" with the volumes of bacteria, buffers and other assay conditions?
A: No. It is extremely important to follow the test protocol instructions to the word. Since the test is very sensitive, any seemingly minor variations result in poor reliability.

Q: ***Can I reuse the provided test vials?***
A: Due to the high sensitivity of the assay, care should be taken to keep all vials, plastic tips, and pipettes extremely clean. Do not reuse test vials and do not wash glassware pipettors or pipette tips with detergent, acid, or solvents.

Q: What is the shelf life of the reagents?
A: The shelf life of the freeze dried bacteria is one year when stored in a deep-freezer (-10°C to -20°C). Reagent should not be stored in a self-defrosting freezer, which defrosts by warming up periodically. In the TOX-SCREEN kit, the assay buffers should be stored in a regular refrigerator (~4°C) and under no circumstances should they be frozen. In the TOX-SPOT kit, the assay buffers are provided in freeze-dried form and should be kept in the freezer under the same conditions as the bacteria.

Q: How do environmental conditions affect the response of the bacteria to toxic chemicals in water?

A: While the optimal temperature for conducting the test is 30°C, the bacteria will respond well in a wide range of temperatures (18°-35°C). One should keep in mind that some chemicals effect bacteria faster than others, especially at sub-mg/L concentrations. As a rule of thumb, the lower the temperature the longer it takes for the assay to reach its maximal sensitivity (especially when testing organic toxicants). Under optimal conditions, an average time of 15 minutes is usually enough to detect most toxicants.

8. Bibliography

Bacteria bioluminescence

http://www.biology.pl/bakterie_sw/index_en.html

http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed&uid=396467&cmd=showdetailview&indexed=google

http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-83822003000200001&lng=in&nrm=iso&tlng=in

Bulich AA, Isenberg DL. 1981. Use of the luminescent bacterial system for the rapid assessment of aquatic toxicity. ISA Transactions, 20: 29-33.

Kaiser KL. 1998. Correlations of Vibrio fischeri bacteria test data with bioassay data for other organisms. Env. Health Perspect. Apr;106 Suppl 2:583-91.

Early warning systems

ASCE- Database of commercially available and Emerging Technology
Water Monitoring Equipment- http://www.asce.org/static/1/redirect.cfm?prmType=WISE&prmFile=1/16_Appendix_File_2_of_3

Using higher organisms in biological early warning systems for real-time toxicity detection - van der Schalie et al .2001. http://cat.inist.fr/?aModele=afficheN&cpsidt=14131451

Strategic monitoring for the European Water Framework Directive - Ian J. Allan et al. Trends in Analytical Chemistry, Vol. 25, No. 7, 2006. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6V5H-4KF7812-1&_user=10&_coverDate=08%2F31%2F2006&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=c2f64138f7df4c45f03eb830810f6100.

Early Warning Monitoring to Detect Hazardous Events in Water Supplies. ILSI report.1999. http://rsi.ilsi.org/Publications/EWM.htm

Water Security Initiative: Interim Guidance on Planning for Contamination Warning System Deployment. http://www.epa.gov/safewater/watersecurity/pubs/guide_watersecurity_securityinitiative_interimplanningpdf.pdf

Technologies and Techniques for Early Warning System to Monitor and Evaluate Drinking Water Quality: State-of-the-Art Review. EPA http://www.epa.gov/nhsrc/pubs/reportEWS120105.pdf

Introduction to EPA'S Drinking Water Source Protection Programs. http://www.epa.gov/watertrain/statemeasures.html

Low-cost ecotoxicity testing of environmental samples using microbiotests for potential implementation of the Water Framework Directive. K. Wadhia, K. Clive Thompson. Trends in Analytical Chemistry, Vol. 26, No. 4, 2007. https://enduser.elsevier.com/campaign/index.cfm?campaign=emerging

Water Monitoring Equipment for Toxic Contaminants Technology Assessment. 2004. http://www.techstreet.com/cgibin/detail?product_id=1233866

Water Safety Plans Managing drinking-water quality from catchment to consumer. WHO. http://www.who.int/water_sanitation_health/dwq/wsp0506/en/index.html

TOX-SCREEN Test
Selection of a battery of rapid toxicity sensors for drinking water evaluation . van der Schalie et al. Biosensors and Bioelectronics 22 (2006) 18–27. http://stinet.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA455071

A Novel and Sensitive Test for Rapid Determination of Water Toxicity. S. Ulitzur, et al. Environmental Toxicology Volume 17, Issue 3 , Pages 291 - 296. 2002. http://www3.interscience.wiley.com/cgi-bin/abstract/95515584/ABSTRACT

ETV verfication – Rapid Toxicity Testing Systems- http://www.epa.gov/etv/verifications/vcenter1-27.html

Biotests and Biosensors in the Analysis of (eco)toxoxicological Risk of Soils Highly Polluted by Cadmium, Lead and Zinc: Bioavailable Fractions cause Toxic Hazard. KAHRU et al. http://www.unece.org/ie/intersol/documents/s48e.pdf

Leachate toxicity. Japan. http://www.cis.fukuoka u.ac.jp/~higuchis/H18 seika/H18.hai 9 E02-11.pdf..

On-line real time monitoring - peace of mind? Schreppel et al. 2003. http://www.ucowr.siu.edu/proceedings/2003 Proceedings/W5C.pdf.

Pilot river basin case study: The Ribble estuary (UK). Ian Allan. 2005.
http://www.gentronix.co.uk/Portals/0/literature/posters/Poster SWIFT Ribble Trial 2005.pdf

SWIFT-WFD- Screening methods for Water data information in support of the implementation of the Water Framework Directive. http://www.swift-wfd.com/

The use of field studies to establish the performance of a range of tools for monitoring water quality. B. Roig et al. Trends in Analytical Chemistry, Vol. 26, No. 4, 2007
http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6V5H-4N2TRX9-2&_user=10&_coverDate=04%2F30%2F2007&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=6840997d85f057fabc0e6834ca6b1cb2

Testing chemicals with low-solubility in water
Jay, A.E., 1996. Bulletin of Environmental Contamination and Toxicology 57:191-198.
Stratton G.W.,1985. Archives of Environmental Contamination and Toxicology 14:651-658.
Stratton G.W. 1989. Bulletin of Environmental Contamination and Toxicology, 42:754-760.
Stratton G.W. and C.T. Corke, 1981. Bulletin of Environmental Contamination and Toxicology 27, 13-16.
Stratton G.W., Burrel R.E., and C.T. Corke. 1982. Archives of Environmental Contamination and Toxicology, 11:437-445.
Stratton G.W., and T.M. Smith.1988. Bulletin of Environmental Contamination and Toxicology, 40:736-742
Ma J and J Chen. 2005. Environmental Pollution 136:267-273.