INDIANA DEPARTMENT OF TRANSPORTATION
DIVISION OF MATERIALS AND TESTS

RECYCLED FOUNDRY SAND TOXICITY TEST
ITM No. 215-15

1.0 SCOPE.

1.1 This test method covers the procedure for the rapid evaluation of the toxicity of recycled foundry sand using luminescent marine bacterium.

1.2 The luminescent marine bacterium, *Vibrio fischeri*, produce light as a result of the normal biological activity for this bacterium. The test exposes the luminescent bacterium to aqueous leachate samples and measures the change in the light output at 5 and 15 minutes. Leachate samples that contain toxins cause a reduction of the light output due to a decrease in the biological activity of the bacterium. By measuring the relative change in the light output of the bacterium exposed to the leachate samples verses the change in the light output of a control sample the relative toxicity of the spent foundry sand may be determined.

1.3 This ITM may involve hazardous materials, operations, and equipment and may not address all of the safety problems associated with the use of the test method. The user of the ITM is responsible for establishing appropriate safety and health practices and to determining the applicability of regulatory limitations prior to use.

2.0 REFERENCES.

2.1 ASTM Standards.

D5660 Test Method for Assessing the Microbial Detoxification of Chemically Contaminated Water and Soil Using a Toxicity Test with Luminescent Marine Bacterium

E943 Terminology Relating to Biological Effects and Environmental Fate

2.2 ITM Standards.

207 Sampling Stockpiled Aggregates

802 Random Sampling

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2.3 Other.

MICROTOX ® 90% comparison test protocol, 1998 AZUR Environmental.

3.0 TERMINOLOGY. Definitions for terms and abbreviations shall be in accordance with the Department's Standard Specifications, Section 101 and ASTM E943.

4.0 SIGNIFICANCE AND USE. The test method is used as an indicator to determine acceptance or rejection of recycled foundry sand from a ferrous foundry for highway construction projects. Spent foundry sand is required to first conform to Indiana Administrative Code, 329 IAC 10, for Type III or Type IV restricted waste. Testing for this typing is accomplished by the foundry using accepted EPA Methods. When re-sampling of the recycled foundry sand is required for waste reclassification, 329 IAC 10-9-4 testing per this protocol is also required.

5.0 APPARATUS. The testing apparatus shall be in accordance with ASTM D5660.

6.0 SAMPLING. Unless otherwise directed, samples shall be obtained as follows:

6.1 Identify the volume and portion of the recycled foundry sand open-stockpile that shall be used on construction projects. Divide this portion of the stockpile into lots of approximately 20,000 yd^3. Divide each lot into five sublots of equal volume.

6.2 Lots of less than 20,000 yd^3, each sublot shall be approximately 4000 yd^3.

6.3 Determine random sampling locations within each sublot in accordance with ITM 802. The depth of the sampling shall never be less than 1 ft or require an excavation greater than 8 ft.

6.4 Sample each sublot in accordance with ITM 207.

6.5 Foundry's that store recycled foundry sand using a method other than an open - stockpile shall provide a QCP which provides for random sampling of each sublot of 4000 yd^3.

7.0 SAMPLE PREPARATION. The leachate sample preparation of each sublot sample for toxicity testing shall be as follows:

7.1 Place 20.00 ±0.05 g of the recycled foundry sand into a flask, add 80 ±1mL of water to the flask. Cover the flask with parafilm, manually agitate to break down any large clumps, and place the flask on a shaker table at 175 to 200 RPM for 18

±2 h. No mechanical breaking or crushing of the sand shall be performed in order to represent actual site conditions.

7.2 After shaking allow the sample to settle, then pour the supernatant into polycarbonate centrifuge tubes and centrifuge for 16 minutes at 10000 RPM. Filter the supernatant using a 1.5 µm pore size glass fiber filter and then a 0.45 µm pore size membrane filter to remove fines. Measure the pH of the filtrate and transfer to borosilicate glass vials, cover with parafilm and cap.

7.3 Each of the leachate samples shall be tested immediately or stored at 39°F for no more than 72 h prior to testing.

8.0 PROCEDURE. The leachate samples shall be tested in accordance with the MICROTOX ® 90% comparison test protocol in appendix A.

9.0 CALCULATION.

9.1 For each leachate sample, calculate a control test mean, Xc, and a sample test mean, Xs, for each time T5 and T15.

9.2 Calculate the normalized mean difference, $\overline{X}$, between the means of the control test and the sample test for each time, T5 and T15, for each sublot sample.

$$\overline{X} = \frac{(X_c - X_s) \text{ x } 100}{X_c}$$

where:

X_c = control test mean
X_s = sample test mean

9.3 Calculate the pooled mean difference from all the samples, $\overline{X}p$

$$\overline{X} = \frac{1}{p}\sum_{i=1}^{p} \overline{X}$$

where:

p = the number of sublot samples
$\overline{X}i$ = the normalized mean difference of the *i*-th sample

9.4 If any $\overline{X}i$ is greater than $\overline{X} + 2\sigma$ □ assume σ □= 3.0, then obtain a new sublot sample and include both new sample values and the original values for $\overline{X_i}$ in the $\overline{X_p}$ calculations.

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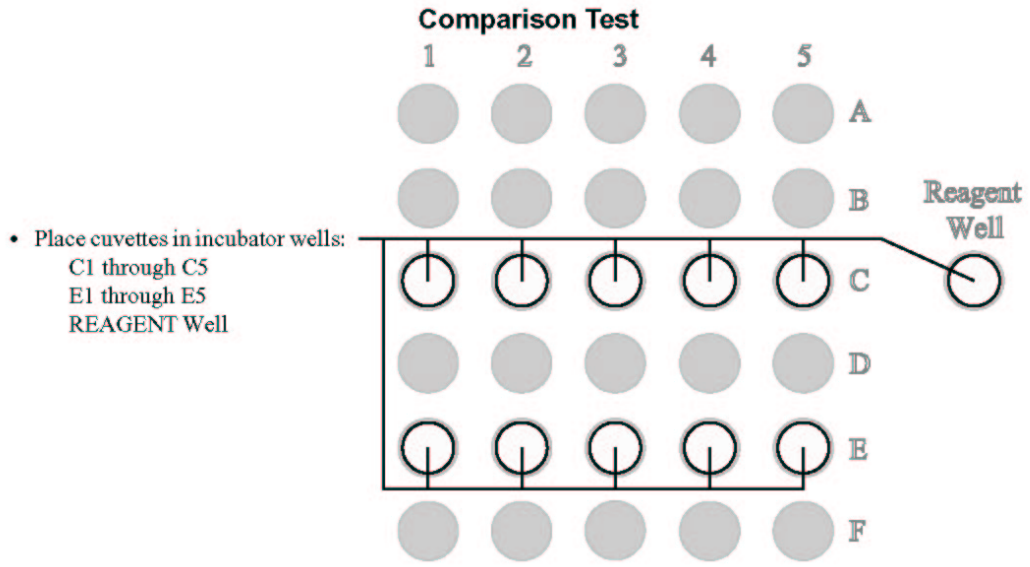
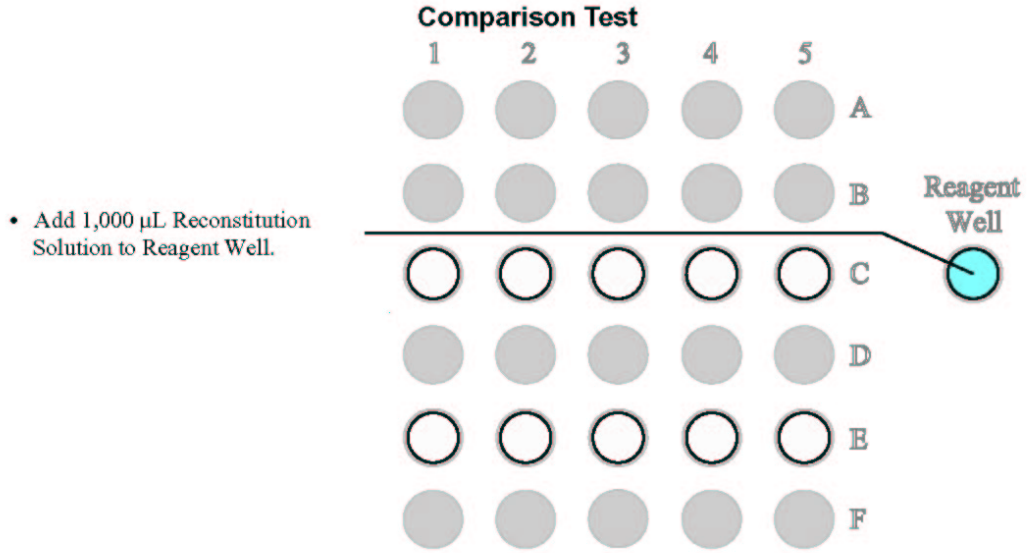
10.0 REPORT. The report of the results shall consist of TCLP and Neutral Leachate Test results upon which the waste classification is based, certification that the ferrous recycled foundry sand is Type III or Type IV as set out in the Recurring Special Provisions for use of recycled foundry sand in construction projects, pH of the leachate from each sample, and the results of the MICROTOX ® 90% comparison test protocol. The MICROTOX ® results shall include T5 and T15 mean values for each of the sublot samples including rejected sublot samples. The dates of the sampling and testing shall be included in the test report. The test results shall be kept on file for a minimum of five years. The report shall be sent to the Environment, Planning and Engineering Division.



Page 1



Page 2

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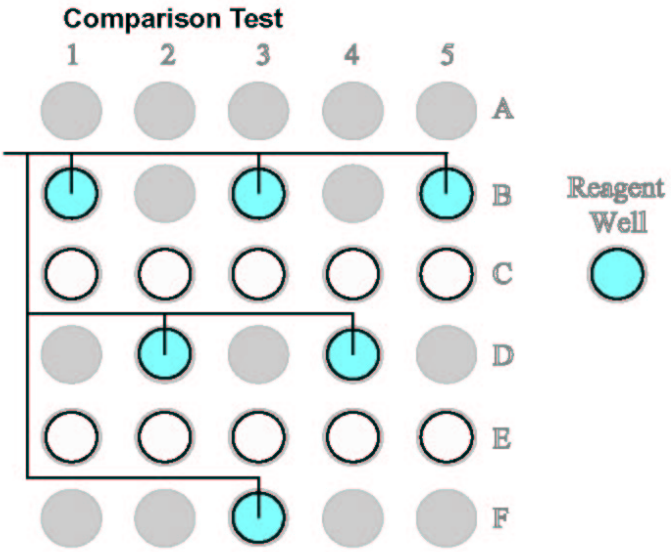
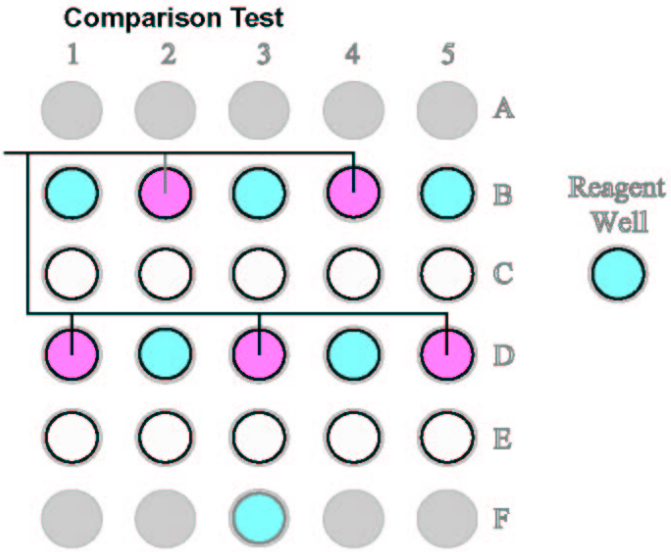
- Add 10.0 mL Reconstitution Solution to a test tube.
- Add 1,000 µL OAS to the test tube, mix.
- Transfer 1,500 µL Osmotically Adjusted Reconstitution Solution to six cuvettes. Place Osmotically Adjusted Reconstitution/cuvettes in wells:
  B1, B3, B5
  D2, D4
  F3



- Add 10.0 mL of sample to a test tube.
- Add 1,000 µL OAS to the test tube, mix.
- Transfer 1,500 µL Osmotically Adjusted Sample to five cuvettes. Place Osmotically Adjusted Sample/cuvettes in wells:
  B2, B4
  D1, D3, D5
- Wait 5 minutes.



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Comparison Test

Reagent Reconstitution

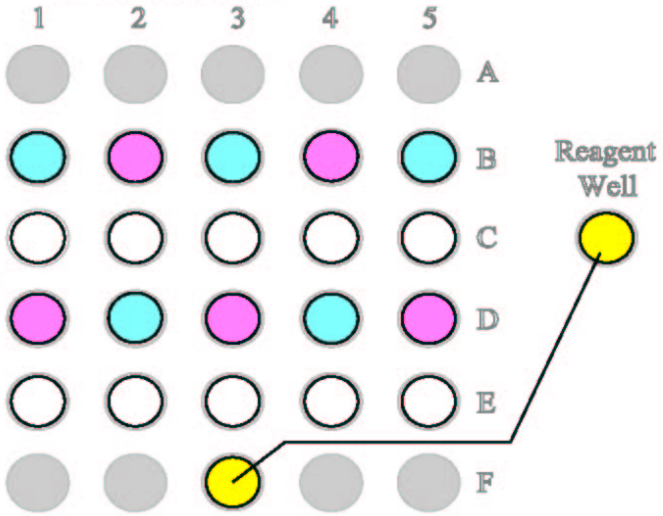
Reconstitute a vial of Microtox Acute Toxicity Reagent in the following way:

- Remove a single vial of reagent from the freezer and open it with the minimum of handling, thereby reducing warming of the vial.
- Shake and tap the vial gently to ensure the pellet of bacteria is seated on the bottom of the vial.
- Take the precooled cuvette of Reconstitution Solution from the Reagent Well, then quickly pour the solution into the opened vial.
- Swirl the vial 3 or 4 times, then quickly pour the mixture back into the cuvette and return it to the Reagent well.
- Mix the bacteria thoroughly using the pipettor by aspirating and dispensing 0.5 ml of solution at least 10 times. Reconstituted bacteria should be used within 3 hours of reconstitution. Further tests after this period require the preparation of freshly reconstituted bacteria.

Page 5

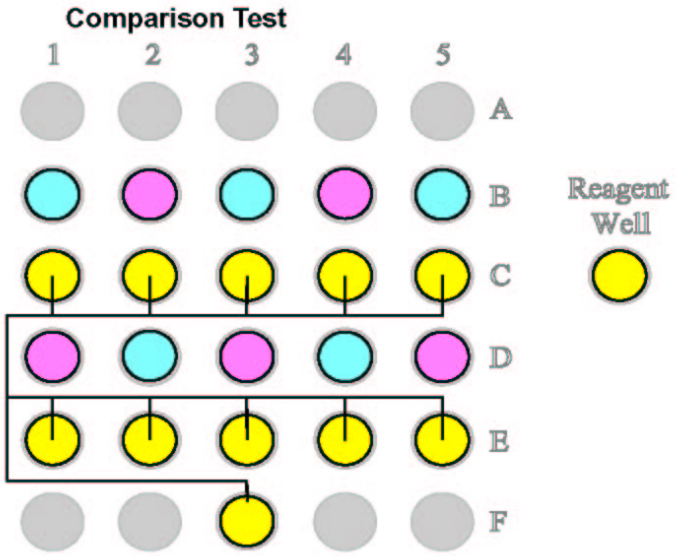
Comparison Test

- Transfer 150 µL reagent to F3, mix.



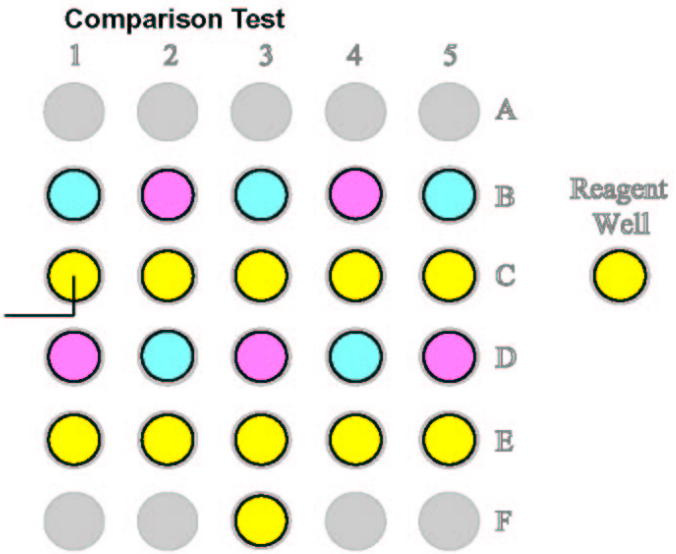
Page 6

- Add 100 µL diluted reagent (F3) to:
  C1 through C5
  E1 through E5
- Wait 15 minutes.



Page 7

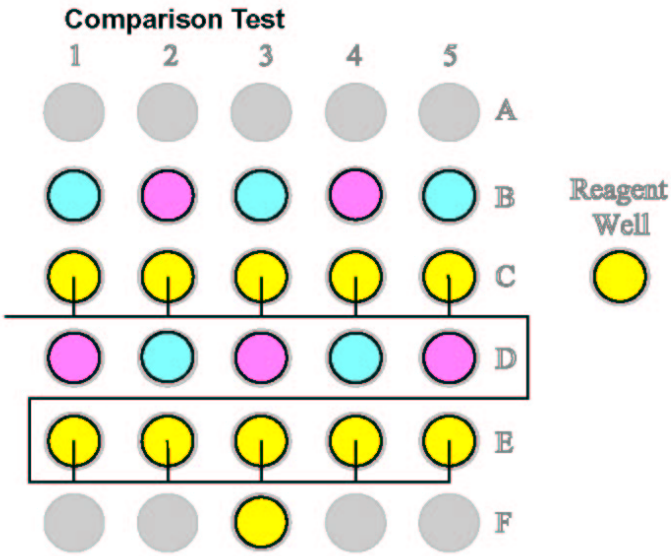
- Place C1 cuvette in READ well. Press the SET button.
- Touch the computer <space bar> key.



Page 8

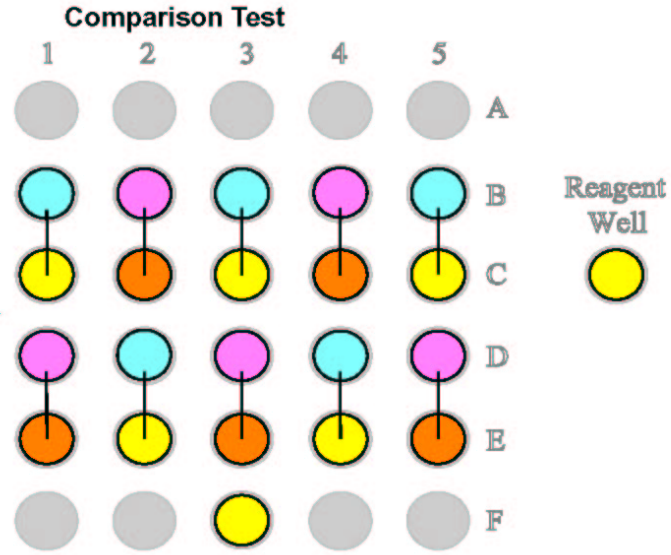
- READ zero time I_o light levels as prompted by the computer monitor:
  C1, C2, C3, C4, C5
  E1, E2, E3, E4, E5

  Then immediately...



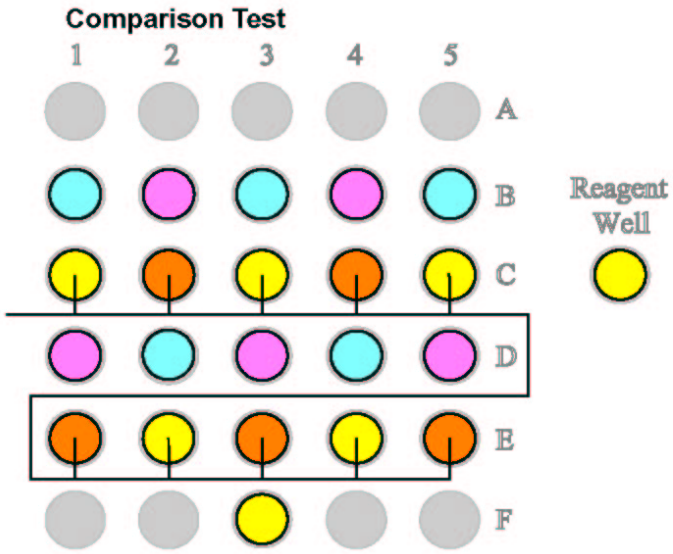
Page 9

- ... make the following 900 µL transfers:
  - B1 to C1
  - B2 to C2
  - B3 to C3
  - B4 to C4
  - B5 to C5
  - D1 to E1
  - D2 to E2
  - D3 to E3
  - D4 to E4
  - D5 to E5
- Touch the computer <space bar> key.



Page 10

- When timer sounds, READ light levels as prompted by the computer monitor:
  C1, C2, C3, C4, C5
  E1, E2, E3, E4, E5



Page 11

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