A Simplified Microtox Bioassay Procedure for Drilling Waste Testing

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ABSTRACT

A Microtox® bioassay is required before drilling waste can be disposed of on land in Alberta. The test has been the regulatory standard for toxicity testing in the Alberta drilling industry for over 15 years. The traditional method involves testing a range of dilutions of a sample of fluid derived from the waste.

The project being reported here concerns an equivalent, simpler version of the test, employing only one dilution level, namely the regulatory Pass threshold level (75 %). The simplified method has advantages of speed and economy, requires less material and supplies, and involves fewer calculations than the traditional method.

In tests with a wide range of drilling waste samples, the two methods picked out exactly the same passing and failing wastes, thus demonstrating the technical equivalence required for a valid alternative method of drilling waste testing.

INTRODUCTION

In a typical week, hundreds of wells are drilled for oil and natural gas in western Canada, mostly in Alberta. The resulting drilling waste – usually composed of water, cuttings, drilling fluid and additives – is often stored temporarily at the surface and disposed of on-site following Directive 50 (ERCB, in press).

Under D-50, before disposal certain wastes must pass the Microtox bioassay, in which four serial dilutions of fluid derived from the waste sample are mixed with luminescent bacteria *(Vibrio fischeri)* to obtain an EC50 value from the resulting light readings. For a Pass result, the EC50 value after 15 minutes' contact (a measure of sample toxicity) must be at least 75 % of the initial fluid concentration.

The alternative, previously untried, of measuring light loss directly at the 75 % threshold level would have the advantage, over the 4 serial dilutions version, that more samples could be tested in one run, using less of the expensive test bacterium. A "one-shot" procedure would also be better suited to on-site screening.

Under D-50, wastes that fail the Microtox test must then be tested for petroleum hydrocarbons (PHC). In the interests of lab turn-around time, PHC measurements are often started before the result of the Microtox test is known; for non-toxic samples, this wasted effort could be avoided if a one-shot Pass result was quickly available.

MATERIALS AND METHODS

Numerous drilling wastes from well sites in Alberta were available for the project, in the form of samples sent to ALS for routine D-50 testing during the period Dec. 2010 – Feb. 2011. The range of sample types – fluids, sump solids and total wastes – was typical of sump material generated using current practices.

As-received fluid, or supernatant fluid from centrifuged over-saturated wastes, or from 1:1 aqueous extracts of denser sludges, was tested by the standard Microtox bioassay, after pH adjustment into the range 6.0-8.8 if necessary (WCMUC 1994).

The increased sensitivity assay used (ISA, commonly employed by testing labs) goes as follows: The test fluid is first made 2 % in NaCl by spiking with osmotic adjustment solution (OAS), then is serially diluted 2-fold in 2 % NaCl (Diluent). Portions (0.90 mL) of each mixture are then added to 0.10 mL aliquots of the test bacterium *Vibrio fischeri* in glass cuvettes at 15 ^{o}C. The ISA concentrations tested are 10.2, 20.5, 40.9 and 81.8 % of the initial sample.

While running the routine ISA, we also tested each fluid after adding 1.00 mL to a cuvette containing 0.10 mL each of OAS (22 % NaCl) and Diluent, thus obtaining an 83.3 % dilution in 2 % NaCl. To start the bioassay, 0.90 mL of that mixture was added to a 0.10 mL aliquot of bacteria, thus giving a test concentration = 75 % exactly.

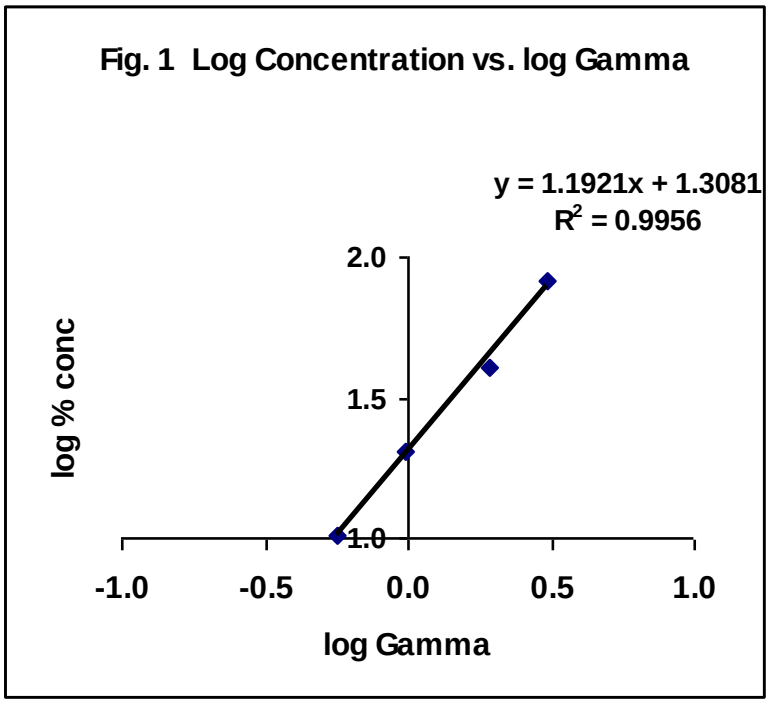
In our one-shot procedure, as in the ISA, 0.90 mL of pure Diluent was also added to an aliquot of bacteria, to control for natural drift in bacterial light output after mixing. In all, 72 samples were analyzed by both methods.

RESULTS AND DISCUSSION

4 serial-dilutions ISA procedure

In the ISA test, the EC50(15) value is obtained from a line of best fit through the 4 data points on a (usually log-log) plot of test concentration vs. the light loss ratio Gamma (defined below).

The EC50(15) value (corresponding to a halving of light intensity after 15 minutes) is the (antilog of the) intercept where Gamma = 1, i.e. where log Gamma = 0. In the toxic example shown (Fig. 1), light loss increased with sample concentration, and the intercept (1.3081) indicated an EC50(15) = 20.3 %.



The higher the EC50 value, the less toxic the sample. The ISA results for the 72 test fluids (Table 1) indicated that 36 of them failed D-50, due to having EC50(15) values < 75 %.

Table 1. Summary of 4-concentration test results

EC50(15)	No. of samples tested	Toxicity Rating
< 25 %	**13**	**Fail (very toxic)**
25-50 %	**9**	**Fail (toxic)**
50-75 %	**14**	**Fail (somewhat toxic)**
75.0-100 %	**7**	**Pass (slightly toxic)**
> 100 percent	**29**	**Pass (non-toxic)**

One-concentration test

In the one-shot test, the EC50(15) of the fluid is not evaluated. The Pass/Fail criterion becomes the value of the light loss ratio Gamma at the single dilution level used (75 %).

Gamma, the ratio of light lost to light remaining after mixing sample and bacteria, is calculated from light intensity readings (I) made at t = 0, 5 and 15 min. (Microbics 1992).

$$\textbf{Gamma} = \textbf{Sample } (I_o - I_t) / I_t = \textbf{Sample } (I_o / I_t) - 1 \qquad \textbf{[Eqn. 1]}$$

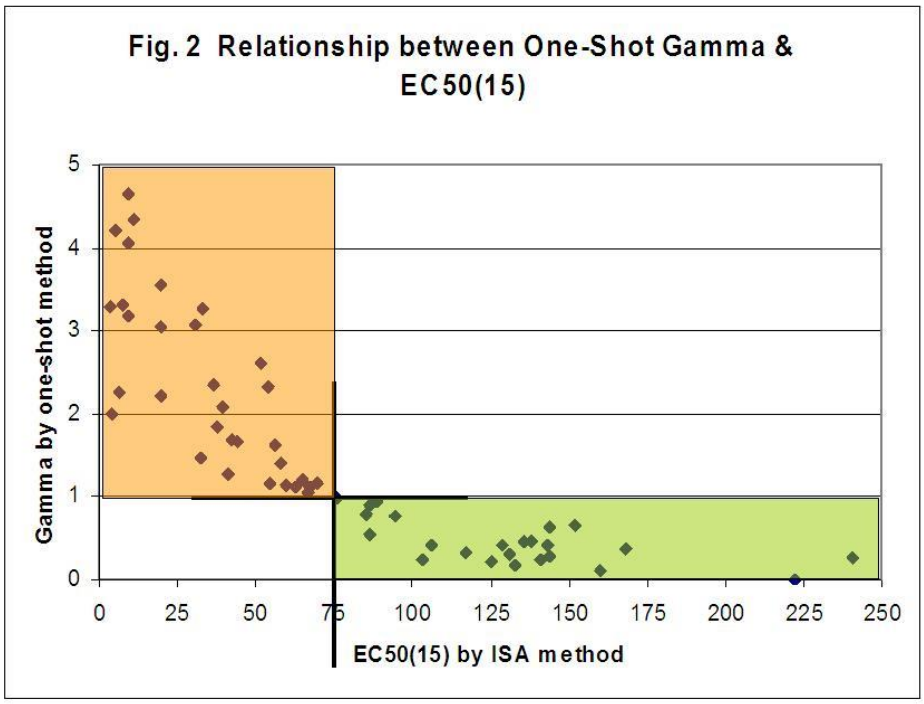
Even if no mixing was done, I_t and I_o would differ due to natural drift in bacterial light output. To obtain the true I_o / I_t ratio due to sample toxicity, parallel readings, made before and after mixing a control aliquot with Diluent solution (pure 2 % NaCl), are used to adjust the sample's I_o (using the second form of Eqn. 1); the correction is usually small.

$$\textbf{Gamma} = [\textbf{Control } (I_{15} / I_o) \times \textbf{Sample } I_o / I_{15}] - 1 \qquad \textbf{[Eqn. 2]}$$

The sample fails D-50 if Gamma exceeds 1.0 (bacterial light more than halved) after 15 min. The one-shot test results and ISA test results are

compared in a plot (Fig. 2) of Gamma against EC50(15). The scatter in data points is caused by variability in the effect of sample concentration on bacterial light intensity (i.e. the slope of Fig. 1) from one test fluid to another. Such scatter makes it impracticable to fit an equation for accurate calculation of EC50(15) from Gamma.

However, from the viewpoint of drilling waste disposal contractors and their clients, the actual EC50(15) value is of less concern than knowing whether or not the value is less than the Pass / Fail threshold of 75 %. By the same token, determining whether or not Gamma is greater than 1.0 meets the main purpose of the drilling waste bioassay.



It will be seen that positioning Cate-Nelson cross-hairs (Tisdale et al. 1993) at Gamma = 1.00 and EC50(15) = 75 % separates all points into opposing quadrants, putting every failing sample in the upper left (red) quadrant and every passing sample in the lower right (green) quadrant.

As expected for a wide range of test samples, a few passed or failed D-50 only narrowly but, even so, the one-shot test identified exactly the same individual passing or failing fluids as the 4 serial-dilutions ISA test.
One-shot Gamma values > 4 indicate very high toxicity; values in the range 2-4 moderate-high toxicity, in the range 1-2 slight-moderate toxicity and values ≥ 1 little or no toxicity. These categories are equivalent to the EC50(15) ranges in Table 1, for assessing the degree of toxicity.

Colour/turbidity correction

In addition to any effects due to fluid toxicity, brown and turbid fluids interfere with light emitted by *Vibrio fischeri*, which at 490 nm is near the blue end of the visible spectrum. The Omni™ colour correction method (Azur 1999) requires a separate spectrometer reading of optical absorbance at 490 nm, which for turbid samples over-corrects Gamma, probably due to light-scattering in the optical cell.
An accurate correction, even for significantly turbid test fluids, is obtained using a modified double cuvette (Ashworth et al. 2010). To obtain the corrected Gamma value, the sample's I_{15} reading used in Eqn. (2) is multiplied by the ratio (Q) of separate light readings made with the outer compartment of the double cuvette either empty, or holding the light-absorbing test fluid.
A volume of 75 % solution sufficient for measuring Q is easily obtained by adding 0.133 mL of extra Diluent to a duplicate 1.200 mL of sample at the 83.3 % level (made as described on p. 3); this volume increase is equivalent to adding 0.90 mL to 0.10 mL of *V. fischeri*. In the double cuvette, the 75 % test solution surrounds rather than mixes with the bacteria, thus avoiding light loss due to toxicity.

Quality Control of Bacteria

Using phenol as a standard toxicant, the intercept at Gamma = 1 (Fig. 1) should fall in the range 13 – 26 mg/L (Microbics 1992). Back-calculation shows that, with 26 mg/L phenol solution, Gamma at the one-shot 75 % concentration should fall in the range 0.70 – 1.30.

Confidence Limits on Gamma values

When Gamma is near 1.0, replicate 15-minute light readings typically agree within ± 2 units, and the resulting uncertainty on Gamma is ± 0.1 for practical purposes. In rare cases, a sample could fail D-50 within that margin of uncertainty but a similar possibility exists in the ISA procedure, due to uncertainty in EC50(15) values.

Values slightly less than 75.0 % are acknowledged as failures by the industry, despite the related uncertainty; similar data interpretation would apply to Gamma values only slightly greater than 1.00.

CONCLUSIONS

For 72 typical drilling wastes, ranging widely in toxicity, the traditional four serial-dilutions (ISA) Microtox procedure and a one-shot 75 % concentration test each singled out the same 36 fluids as sufficiently toxic to fail D-50. This finding therefore demonstrate the equivalence required in D-50 for validating alternative methods of drilling waste testing.

Light loss ratio (Gamma) values from the one-shot test give an accurate indication of the degree of sample toxicity.

As in the ISA procedure, data from the one-shot test can easily be corrected for sample colour and turbidity if necessary, and QC is likewise readily

maintained using blanks, duplicates and phenol solution as a toxicity standard.

ACKNOWLEDGEMENTS

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