

Toxicity Testing of Municipal Solid Waste Leachates with CerioFAST

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Disposal in sanitary landfills remains the primary method of managing solid waste generated by residential and commercial activities, also known as municipal solid waste (MSW) (USEPA, 1996). Contact between rain water and the landfilled waste results in a wastewater referred to as leachate. Leachate may contain a vast array of different organic and inorganic chemicals. Current United States regulations require that landfills be lined to intercept and remove the leachate before environmental contamination can occur (CFR, 1996). The typical method of leachate management involves treatment at a domestic wastewater treatment facility.

Current research supports the use of sub-lethal endpoints in toxicity testing. Microbiotests, using invertebrates as the test organisms, and their application to aquatic toxicology, have been reviewed recently (Janssen, 1997; Persoone, 1998). These include cyst-based 24- to 48-hr tests (e.g., Toxkits) and others based on *in vivo* inhibition of enzyme (e.g., beta-galactosidase, esterase) activity. More rapid invertebrate tests are based on inhibition of feeding activity by toxicants. The latter include a rotifer test based on inhibition of feeding on fluorescent beads (Juchelka and Snell, 1995) and a daphnid test based on suppression of feeding activity, using fluorescently-labeled yeast cells as food (Bitton et al., 1995, 1996). There is, however, little data on the application of these short-term (i.e., 1-2 hr) invertebrate tests to field samples. Earlier research by Bitton et al. (1996) and Jung and Bitton (1997) established a significant correlation between 1-hr CerioFAST and the standard 48-hr *Ceriodaphnia dubia* acute toxicity test, using both pure compounds and field samples of industrial effluents.

The objective of this research was to increase the database of EC₅₀ (concentration which effects 50% of the population) results for 1-hr CerioFAST, with other types of effluents. Simultaneously, we reaffirmed the correlation previously shown (Jung and Bitton, 1997) between the 48-hr *C. dubia* acute toxicity test and the 1-hr CerioFAST using municipal solid waste (MSW) landfill leachates.

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MATERIALS AND METHODS

Two acute toxicity tests, 1 -hr CerioFAST and the 48-hr *Ceriodaphnia dubia* acute toxicity test, were used to assess the toxicity of MSW landfill leachates in three Florida counties.

The Alachua County Southwest Landfill (ACSWL) is located west of Archer, Florida on S.R. 24 in Alachua County and has been in operation since 1973. The Central Landfill (CL) is located west of Inverness, Florida on S.R. 44 in Citrus County and has been in operation since 1975. The Levy County Landfill (LCL) is located 4 miles northeast of Bronson, Florida and has been in operation since 1975. Further descriptions of the landfill sites are available (Ward, 1997).

C. dubia cultures were kindly supplied by Hydrosphere Research (Gainesville, FL). The test organisms were cultured according to modifications of U.S EPA methodology (1991).

The CerioFAST acute toxicity assay has been previously described (Bitton et al., 1996; Jung and Bitton, 1997). Briefly the test is based on the suppression of feeding activity by a toxicant. Neonates (<24 hours old) were isolated and held in clean moderately hard water (MHW) two hours prior to the initiation of the assay. MHW was used as a negative control and for preparation of sample dilutions. Ten neonates were placed in empty 30-mL plastic cups to allow for the removal of excess culture water. A 20-mL aliquot of the sample, or dilutions thereof, was placed in the cups. All tests were run in triplicate with appropriate controls. The sample cups were placed in a water bath and maintained at $20 \pm 2^{\circ}\text{C}$. Following 1-hr exposure to a given sample, the daphnids were fed 150- μL of DTAF-stained yeast cells and were allowed to feed for 20 minutes (Bitton et al., 1995). The 5-(4,6-dichlorotriazin-2-yl)-aminofluorescein (DTAF) stained yeast cells were prepared according to Bitton et al. (1995). Neonates were removed from the test vessels and placed on a glass slide. The slide was then observed with an epifluorescent microscope (Nikon, Japan) with a mercury lamp (Nikon model HB-10101AF, Japan) at 100X. Neonates not inhibited by the sample would uptake the fluorescent food resulting in gut fluorescence. No distinction was made between full guts and partially full guts. Neonates with non-fluorescing guts were considered to be inhibited by the sample. Mortality greater than 10%, in the controls, negated the test. CerioFAST results are expressed as EC_{50} , the concentration of sample which caused an effect (e.g., failure to uptake food, or reduced food uptake rate) in 50% of the test organisms.

The standard 48-hr acute toxicity test is a method currently used by the U.S EPA (1991) and APHA (1989). The test endpoint is death/immobilization of neonates (<24 hour old) following a 48-hour exposure to a sample. Results are expressed as LC_{50} , the concentration of sample, which caused 50% mortality of the test organism. MHW was used as the negative control and dilution water for all tests. Neonates (<24 hours old) were fed prior to use in the test. Neonates were placed in 30-mL plastic cups with minimization of culture water transfer. A 20-mL aliquot of each test dilution was added to the cups. The cups were placed in a water bath ($20 \pm 2^\circ \text{C}$) for 48 hrs. Neonates were exposed to ambient lighting and were not fed during the test. Neonates were evaluated for death/immobilization by swirling the cup and watching for neonates with the power to swim away from the center. Mortality greater than 10% in the controls negated the test.

RESULTS AND DISCUSSION

MSW leachates from three Florida counties (Alachua, Citrus, and Levy counties) were tested for acute toxicity, using both the 48-hr *Ceriodaphnia* toxicity test and the 1-hr CerioFAST test.

The 1-hr CerioFAST and the 48-hr acute toxicity test results were compared for identical sampling events (Table 1). Alachua County MSW leachates were the most toxic of the three counties. They showed 48-hr LC_{50} 's varying from 1.7 to 3.6%, whereas the 1-hr CerioFAST gave EC_{50} ranging from 3.4 to 7.3%. The toxicity of the Levy County leachates was slightly lower as the LC_{50} ranged from 2.7 to 12.5% and the EC_{50} varied between 10.3 and 24%. The Citrus County leachates were the least toxic of the three counties investigated, with LC_{50} results from 2.4 to 20.9% whereas the EC_{50} varied from 13.5 to 90.7%. A Student's t-Test showed that the Alachua County leachates were more toxic than either the Citrus County ($p=0.001$) or the Levy County ($p=0.002$) leachates. The results obtained fall within the range of toxicity values obtained by other investigators who used fish, invertebrates, algae, or aquatic plants for testing toxicity of MSW leachates (Cameron and Koch, 1980; Clement and Merlin, 1995; Plotkin and Ram, 1984).

An examination of the toxicity data shows that the EC_{50} values given by CerioFAST were consistently higher than the LC_{50} values given by the traditional 48-hr acute toxicity test.

Although the 1-hr CerioFAST generally showed lower toxicity than the 48-hr acute toxicity test, a linear regression of the 48-hr acute toxicity test versus 1-hr CerioFAST produced a correlation ($r^2 = 0.82$) indicating a significant relationship between the two tests (Figure 1).

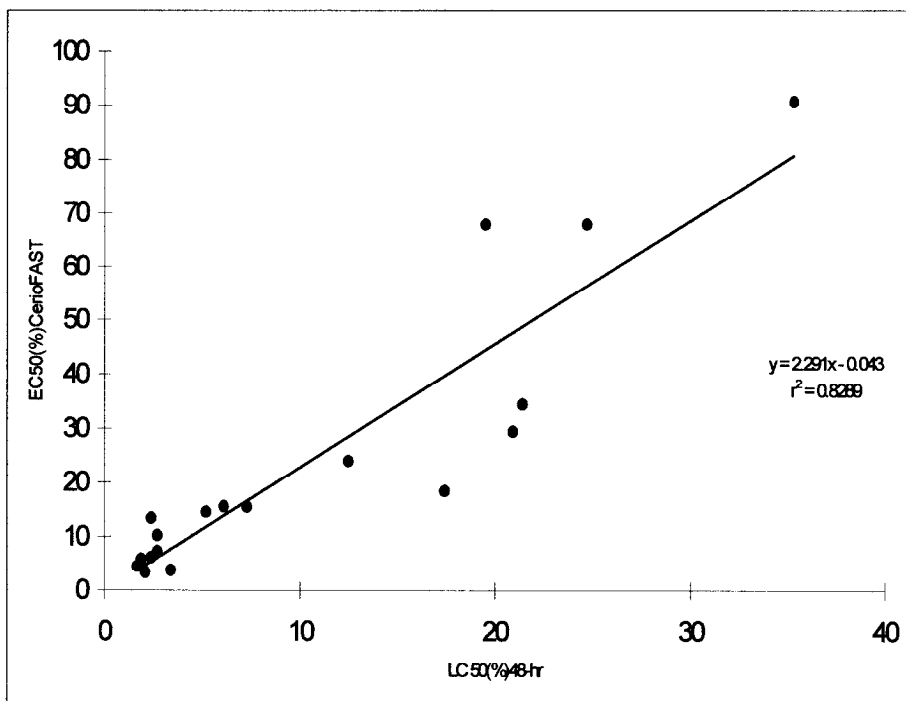


Figure 1. Correlation for the 1-hr CerioFAST and the 48-hr acute toxicity test.

Bitton et al. (1996) determined the toxicity of pure compounds, which included five heavy metals and five organic toxicants, and demonstrated a strong correlation ($r^2 = 0.89$) between the 1-hr CerioFAST and the 48-hr acute toxicity test. The tests displayed comparable EC_{50} values for each of the metals tested, except for silver. However, the comparison of EC_{50} values for the organic toxicants showed a weaker relationship. Jung and Bitton (1997) in a study of industrial effluents showed a correlation ($r^2 = 0.98$) between the 1-hr CerioFAST and the 48-hr acute toxicity assay. Of 29 industrial effluents studied, 23 displayed varying degrees of heavy metal toxicity. The predominance of samples with heavy metal toxicity in Jung and Bitton (1997) study may explain the higher correlation obtained between the 1-hr CerioFAST and the 48-hr acute toxicity test. Ward (1997) determined the heavy metal toxicity of the same MSW leachates, using MetPLATE™, a toxicity test that is specific for toxic metals (Bitton et al., 1994). It was found that most of the samples were non-toxic or displayed low heavy metal toxicity (Ward, Bitton, and Townsend, manuscript in preparation). This suggested the toxicity shown by CerioFAST and the 48-hr acute *Ceriodaphnia* test was due to organic toxicants. Results obtained from this research and that of Bitton et al.

Table 1. Toxicity of Municipal Solid Waste Leachates, using 1-hr CerioFAST and 48-hr *Ceriodaphnia dubia* acute toxicity tests.

Sampling Date	48-hr LC ₅₀ (%)* ± S.D.	CerioFAST EC ₅₀ (%)* ± S.D.
ALACHUA COUNTY		
5-15-97	1.7 ± 0.1	4.5 ± 0.2
5-28-97	3.4 ± 0.3	3.8 ± 0.4
6-23-97	2.1 ± 0.1	3.4 ± 0.6
7-15-97	2.7 ± 0.2	7.3**
8-8-97	2.4 ± 0.1	6.2 ± 1.0
9-2-97	1.9 ± 0.1	5.9 ± 0.3
CITRUS COUNTY		
5-20-97	17.4 ± 0.9	18.5**
6-16-97	2.4 ± 0.3	13.5 ± 0.3
8-4-97	5.2 ± 0.2	14.6 ± 3.0
8-5-97	20.9 ± 2.9	29.5 ± 8.1
8-12-97	24.7 ± 2.7	67.9 ± 4.9
8-19-97	35.3**	90.7 ± 9.8
8-20-97	21.4 ± 1.1	34.5 ± 1.4
9-4-97	19.5 ± 4.3	67.9 ± 4.9
LEVY COUNTY		
6-13-97	7.3 ± 1.1	15.5 ± 0.9
7-15-97	12.5**	24**
8-26-97	6.1 ± 0.4	15.6 ± 3.6
8-26-97	2.7 ± 1.2	10.3 ± 1.8

* Each toxicity test was carried out in triplicate

**standard deviation not available

(1996) and Jung and Bitton (1997) indicated a decreased sensitivity of the 1-hr CerioFAST to organic toxicity when compared to heavy metal toxicity. Organic toxicants may require a longer exposure (e.g., 2-3 hrs) time than heavy metals and this is currently being explored.

In conclusion, although less sensitive, the 1-hr CerioFAST assay offers some advantages over the 48-hr acute toxicity test. The 1-hr CerioFAST assay would provide a rapid assessment of sample toxicity, thereby decreasing the expense and time investment associated with longer

assays (Bitton et al., 1996). It appears to be quite suitable for range finding in toxicity tests, and is now routinely used in our laboratory for that purpose. It would be useful as a screening test prior to allowing the release of truckloads of wastes of unknown toxicity into a given wastewater treatment plant.

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