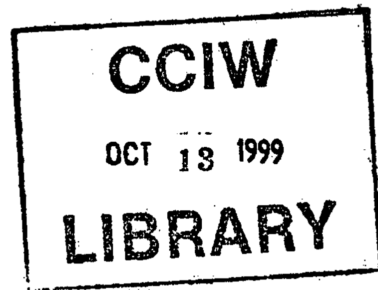
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COMPARISON OF SOLID PHASE TESTING PROCEDURES: DIRECT SEDIMENT TOXICITY TESTING AND MICROTOX SOLID PHASE

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MANAGEMENT PERSPECTIVE

Toxicity testing of solid phase samples (sediments, suspended sediment, soils and sludges) is becoming increasingly important in environmental studies. In the screening of solid phase samples for toxicants, the majority of bioassays used are applied to aqueous or organic extracts of these samples. However, it is often difficult to detect the presence of toxicants in extracts due to their low concentrations and the necessity of diluting extracting solvents to their Maximum Allowable Concentrations (MAC). The effectiveness of these bioassay tests is often nullified by the frequent reports of negative or non-toxic responses mainly due to concentration and dilution problems. Synergistic and perhaps antagonistic responses between toxicants, solvents and extracting/concentrating processes also may play important roles whenever samples are manipulated for bioassay testing. Many bioassays using benthic organisms e.g. *Chironomus tentans* or soil organisms e.g. *Eisenia andrei* to screen solid phase samples for toxicants are usually cumbersome, time consuming and expensive.

An awareness of these problems has led to research and development of simple, quick and inexpensive direct solid phase toxicity testing procedures. Recently, two non-extractive solid phase testing procedures, Microtox Solid-Phase Test and the Direct Sediment Toxicity Testing Procedure (DSTTP), have been developed for testing the toxicity of solid phase samples. These tests can detect the overall toxicity of soluble and insoluble, organic and inorganic, volatile and non-volatile contaminants in solid phase samples without distorting the results due to chemical manipulations or

solvent synergisms. This paper presents the results from the applications of these tests to a variety of samples.

ABSTRACT

Two direct solid phase toxicity testing procedures, Direct Sediment Toxicity Testing Procedure (DSTTP) and the Microtox Solid-Phase procedure were evaluated. Both procedures are practical, simple, rapid, inexpensive and can be used to screen solid phase samples (sediments, suspended sediments, soils and sludges) for the bioavailability of organic and inorganic contaminants in the environment. These procedures are useful in monitoring sediments, landfill sites and effluent streams from biological treatment plants as well as air samples. These toxicity testing procedures could provide sewage treatment plant operators with rapid and sensitive means of assessing the toxicity of solid wastes (sludges) prior to their disposal.

INTRODUCTION

The application of biological and microbiological tests to evaluate the bioavailability of toxicants in environmental solid phase samples (sediments, suspended sediments, soils, and sludges) has significantly increased during the last decade (Dutka and Gorrie, 1989; Tung et al., 1991; Brouwer et al.,1990). In the screening of solid phase samples for toxicants, the majority of bioassays used are applied to aqueous or organic extracts of these samples (Bitton and Dutka, 1986). However, it is often difficult to detect the presence of toxicants in extracts due to their low concentrations and the necessity of diluting/extracting solvents to their Maximum Allowable Concentrations (MAC) (Kwan and Dutka, 1984, 1986 and 1990; Dutka et al. 1989). The effectiveness of these bioassay tests is often nullified by the frequent reports of negative or non-toxic responses mainly due to concentration and dilution problems. Synergistic and perhaps antagonistic responses between toxicants, solvents and extracting/concentrating processes also may play important roles whenever samples are manipulated for bioassay testing. Also, bioassays using benthic organisms e.g. *Chironomus tentans* or soil organisms e.g. *Eisenia andrei* to screen solid phase samples for toxicants are usually cumbersome, time consuming and expensive (Wiederholm,1984; Dutka,1989) .

An awareness of these problems has led to research and development for simple, quick and inexpensive direct solid phase toxicity testing procedures. Recently, two non-extractive solid phase testing procedures, Microtox Solid-Phase Test (Microbics, 1991) and the DSTTP (Kwan, in press), have been developed for testing the toxicity of solid phase samples. These tests can detect the overall toxicity of soluble and insoluble, organic and inorganic, volatile and

non-volatile contaminants in solid phase samples without distorting the results due to chemical manipulations or solvent synergism. This paper presents the results from the application of these tests to a variety of samples.

METHODS AND MATERIALS

Sample Collection

Eight sediment samples were collected from Hamilton Harbour (Lake Ontario). This is a heavily industrialized harbour which receives organic and inorganic contaminants from surrounding industries, including Canada's two largest steel producing companies. Four sediment samples were also collected from the lower Athabasca River in northeastern Alberta. This area contains an extensive oil sand deposits.

The sediment samples were collected with an Ekman dredge and were placed into individual sterile plastic bags, iced and returned to the laboratory for toxicity screening tests.

In addition, 1 uncropped soil sample (WTC-13), 1 cropped soil sample spiked with 10 mg/Kg of PCB (WTC-14), 1 pulp and paper mill anaerobic sludge (WTC-15), 1 incinerator ash (WTC-16), and 4 metal finishing sludges (WTC-17, WTC-18, WTC-19 WTC-20) were also used in this study. These samples were supplied by the Wastewater Technology Centre, Canada Centre for Inland Waters, Burlington.

Direct Sediment Toxicity Testing Procedure (DSTTP)

The direct sediment toxicity testing procedure was used as described by Kwan (1992). The procedures were as follows: set up a series of test tubes (15.5 X 5.6 mm) in a test tube

rack and label them from 1 to 7. Prepare a bacterial suspension by mixing one vial of lyophilized bacteria, Escherichia coli (purchased from Orgenics Ltd.), with 10 mL of filter sterilized LB medium (Bacto trytone 10g; Bacto yeast extract 1g; sodium chloride 10g; D.H2O 1L). Incubate the bacterial suspension at room temperature (20 - 24°C) for 20 minutes before transferring 700 μL into 9300 μL of filter sterilized Reaction Mixture (sodium chloride 12g; potassium chloride 3.7g; sodium dihydrogen orthophosphate-monobasic 2.8g; Bacto trytone 3g; Bacto yeast extract 1.5g; isopropyl ß-D-thiogalactopyranoside 0.14g; D.H2O 1L, pH=7.5). Incubate the bacteria-Reaction Mixture at room temperature for 20 minutes before dispensing 1000 μL into the 1st and 7th previously labelled test tubes, and 500 μL into the remaining tubes (2-6). Add 0.5 gm of sediment, ash, soil or sludge sample into the first tube and mix thoroughly with a vortex mixer for 5 seconds. Transfer 500 μL of the bacteria-sediment mixture into the 2nd tube, vortex and transfer 500 μL to the 3rd tube, continuing the process to tube 6. After vortexing contents in tube 6, 500 μL of mixture is discarded. Before each transfer the bacteria-sediment mixture is vortexed for 5 seconds. 500 μL of Milli-Q water is pipetted into the 7th tube, vortexed for 5 seconds and 500 μL of the mixture is withdrawn and discarded. This tube is the negative control. The tubes are then incubated for 2 hours at 35°C. While the tubes are incubating, place a Whatman GF/F glass microfibre filter (42.5 mm) into petri dishes (50 X 9 mm) which are labelled corresponding to the tubes (Figure 1b). Thirty minutes before the two hour incubation is completed, prepare the yellow chromogenic substrate by mixing a bottle of yellow chromogen with a bottle of yellow chromogen diluent (Orgenics Ltd.,Israel). Then pipet 750 μL of the yellow chromogenic substrate onto each microfibre filter and replace the petri dish lids to prevent drying and leave at room temperature. Each glass microfibre filter can be used to test a maximum of four samples or one sample plus three dilutions (Figure 1b).

After the 2 hour incubation, each tube is vortexed for 5 seconds before 20 μL from each tube is pipetted onto the glass microfibre filters containing yellow chromogenic substrate. Cover the petri dishes and incubate the inoculated glass microfibre filters at 35°C for 30 minutes. After the 30 minute incubation, check for yellow colour development under and around the samples. If the sample is toxic, there will be no yellow colour development. If the sample is non-toxic, a yellow colour will be present around and under the samples (Figure 1a). Based on the intensity of the yellow colour zone compared to the negative control, a semi-quantitative assessment of the bioavailability of toxicant(s) in the sample can be calculated.

Toxicity Interpretations

There are four categories of colour reactions: (1) no yellow colour development, high toxicity level, (-); (2) less than 50% of yellow colour intensity as compared to the control, moderate response, (+); (3) less than 100% but greater than 50% of colour intensity as compared to the control, low response, (++); and (4) yellow colour intensity is equivalent to the negative control, non-toxic, (+++). The more yellow colour developed the less toxic the sample.

Microtox Solid-Phase Testing Procedure

The Microtox Solid-Phase testing procedure was used following the procedure described by Tung, et. al, 1991 and 1991a. The solid phase diluent/reagent mixture is prepared by adding a vial of reconstituted Microtox reagent (bacteria) into a bottle of solid phase diluent. Pipet 2000 μL of the solid phase diluent/reagent mixture into test tubes 1 to 9 and 4000 μL in a test tube labelled #10. Weigh 0.4gm of sediment and transfer it into test tube #10.

Vortex for 5 seconds and make two-fold serial dilutions by transferring 2000 μL mixture from test tube #10 to #9, #9 to #8 .... #3 to #2. After mixing contents in tube #2 discard 2000μL from test tube #2. The #1 tube serves as the blank control. Incubate the bacteria and sediment for 20 minutes at room temperature. After the 20 minute incubation period, a filter column is inserted into each test tube and pushed gently downward to obtain the filtrates. Transfer 500 μL of filtrate from each test tube into its corresponding cuvette in the Microtox incubator block. Incubate 5 minutes at 15°C, then measure the light levels with the Microtox Toxicity Meter (Model M500).

RESULTS AND DISCUSSION

Table 1 presents data obtained from solid phase samples using the Direct Sediment Toxicity Testing Procedure (DSTTP) and the Microtox Solid-Phase toxicity testing procedure. Data obtained from the DSTTP are expressed as minimum concentration of sample (%) that inhibits 100% production of ß-galactosidase activity measured by yellow colour development. Data obtained from the Microtox Solid-Phase Procedure are expressed in EC50s. EC50 is defined as the effective concentration of a test sample that causes 50 percent decrease in light output (Qureshi, et. al 1984). Due to the difficulty of finding "CLEAN" or reference soils, the "CLEAN" soil used in the study was provided by the Microbics Corporation. The "CLEAN" soil is a synthetic soil sample prepared by the U.S. EPA and has an EC50 value > 19,000 ppm. A "CLEAN" or uncontaminated soil sample is defined as a reference soil which has an EC50 value at or above 2% using the Microtox Solid-Phase toxicity testing procedure (Tung et al., 1991).

In this study, any sample with an EC50 value greater than this threshold value of 2% is considered to be relatively non-toxic.

Comparison of the results obtained from the two direct solid phase testing procedures are presented in Table 1. The DSTTP results presented are based on concentrations of samples that produce 100% toxic response i.e. no ß-galactosidase production or activity thus no yellow colour development. On the other hand, the Microtox Solid-Phase results are based on concentrations of samples that inhibit 50% of light production i.e. an EC50 value. In this reporting format the DSTTP results are based on a greater toxic effect and thus a greater concentration of sample is required to produce this effect (100% inhibition) as compared to the EC50 value of the Microtox Solid-Phase test. If the concentration of sediment producing less than 50% yellow colour intensity (+) end point was chosen for comparison, it would be found that the concentration would usually be at least one dilution lower e.g <3.13 = <1.56%, 25% = 12.5% and so on. It can be seen that only one sample, WTC-15, was non-toxic using the DSTTP and there were six negative samples (HH-7, AR-10, WTC-13, WTC-14, WTC17 and WTC-18) using the Microtox Solid-Phase procedure. The most toxic samples based on the Microtox Solid-Phase procedure were WTC-16 (0.03%), WTC-20 (0.04%), WTC-19 (0.05%) and HH-3 (0.06%). Similarly, these same samples were also among the most toxic by the DSTTP e.g. WTC-16 (3.13%), WTC-20 (3.13%), WTC-19 (3.13%), and HH-3 (<3.13%). However, there were five other samples within these same toxic concentration ranges (<0.313% to 3.13%) and they were HH-1, HH-2, HH-4, HH-5 and HH-6. The minor differences in sensitivity between the two tests are possibly explainable by their different indicator systems. In the DSTTP it is believed that the toxic results we observed are due to the inhibition of ß-galactosidase biosynthesis while in the Microtox Solid-Phase test the observed toxic results are based on the inhibition of

luminescence. Earlier Dutton et. al 1988 noted that ß galactosidase activity is inhibited only by heavy metals while ß-galactosidase biosynthesis is inhibited by both heavy metals and organics. Similarly, the Microtox test responds to both heavy metals and organics, but not necessarily to the same degree as the ß-galactosidase inhibition (Dutka and Kwan, 1984; King, 1984).

We surmise that in the one negative DSTTP sample (WTC-15), an anaerobic sludge, from a pulp and paper mill waste, contained few (if any) heavy metals and the organic contaminants combined with the anaerobic condition did not produce sufficiently toxic conditions to trigger a positive effect in the DSTTP. However, these conditions were more deleterious to the Microtox test and registered as a toxic effect. Both the DSTTP and Microtox Solid-Phase procedures indicate that one of the most toxic samples was the WTC-16 sample, an incinerator ash from a plant burning hazardous wastes. These results suggest that a greater effort should be made to evaluate all incinerator ashes for their content of bioavailable toxicants before they are disposed into sanitary landfill sites. The Athabasca River samples, whose main contaminants are believed to be organic in nature from the nearby oil sands and extraction plants and upstream pulp and paper mill effluents, indicate that the DSTTP is equally sensitive in testing for the bioavailability of organic toxicants as it is for heavy metal toxicants as noted in samples WTC-17, WTC-18, WTC-19 and WTC-20. Interestingly both soil samples (with and without PCB) were negative in the Microtox Solid-Phase procedure while producing a strong toxic response in the DSTTP.

Aside from the differences between the two toxicity testing procedures i.e. enzyme production inhibition versus luminescence inhibition and sample testing organism contact time 120 minutes (DSTTP) versus 25 minutes (Microtox Solid-

Phase), a major variation between these tests is that the DSTTP tests a maximum sample concentration of 50% while the Microtox Solid-Phase procedure tests a maximum sample concentration of 10%. This factor may help explain, in spite of testing for an EC100 effect, the fewer negative responses elicited by the DSTTP as compared to the Microtox Solid-Phase testing procedure. Table 2 presents a brief summary comparison of the two procedures.

In conclusion, this study has shown that the DSTTP and the Microtox Solid-Phase testing procedures are practical, rapid, simple and inexpensive procedures to screen solid phase samples for the bioavailability of organic and inorganic contaminants. These solid phase toxicity testing procedures would be extremely useful in the monitoring of sediments, landfill sites, effluent streams from biological and chemical treatment plants as well as collected air samples. These procedures could provide sewage treatment plant operators with rapid, sensitive means of assessing the toxicity of solid wastes(sludges) prior to their disposal.

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TABLE 1: Toxicity data obtained from sediments using the DSTTP and the Microtox Solid-Phase Procedure.

SAMPLE #	DSTTP Sample concentration (%) required to inhibit 100% β-galactosidase production	MICROTOX SOLID PHASE Sample concentration (%) required to produce EC50 effect
HH-1	<3.13%	1.30%
HH-2	<3.13%	0.21%
HH-3	<3.13%	0.06%
HH-4	<3.13%	0.16%
HH-5	<3.13%	0.15%
HH-6	<3.13%	0.20%
HH-7	12.5%	4.11%
HH-8	12.5%	0.67%
AR-9	100%	0.74%
AR-10	12.5%	2.45%
AR-11	50%	0.68%
AR-12	12.5%	1.53%
WTC-13	25%	3.26%
WTC-14	25%	3.07%
WTC-15	NEG	0.96%
WTC-16	3.13%	0.03%
WTC-17	12.5%	3.79%
WTC-18	25%	7.56%
WTC-19	3.13%	0.05%
WTC-20	3.13%	0.04%

HH Hamilton Harbour
AR Athabasca River
WTC Wastewater Technology Centre

TABLE 2: Comparison of DSTTP and the Microtox Solid-Phase Procedure

	DSTTP	**SOLID-PHASE**
Cost per sample (based on a kit & disposal materials)	$46.00	$45.00
Sample size	0.5gm	0.4gm
Highest Test Conc.	50%	10%
Interferences	None	Colour, Turbidity (Colour correction needed)
Instrument	35°C incubator Vortex(option)	Microtox M500 Printer option) Computer (option)
Cost of instrument	Low ($500)	High ($25000)
Bacteria	Freeze-dried (engineered)	Freeze-dried (non-engineered)
Incubation Temp.	Room Temperature 35°C	Room Temperature 15°C
Assay Time	3 hours	1 hour
Type of test	Semi-quantitative	Quantitative
Sensitivity	High	Moderate
Endpoint	ß-galactosidase inhibition	Luminescence inhibition
Labour	minimum	moderate