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Evaluation of Toxi-Chromotest Direct Sediment Toxicity Testing Procedure and Microtox Solid-Phase Testing Procedure

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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 increased significantly 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 1991), 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.

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METHODS AND MATERIALS

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 additional sediment samples were also collected from the lower Athabasca River in northeastern Alberta. This area contains an extensive oil sands deposit.

The sediment samples were collected with an Ekman dredge and 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 used in this study. These samples were supplied by the Wastewater Technology Centre, Canada Centre for Inland Waters, Burlington.

The direct sediment toxicity testing procedure (DSTTP) was used as described by Kwan 1991. The DSTTP is a toxicity bioassay used to detect toxic contaminants in solid phase environmental samples such as sediments, suspended sediments, soils, and solid wastes. A bacteria reaction mixture is mixed with 0.5 gm of sediment and incubated at 37°C for 120 minutes. After a 120 minute incubation period, the mixture is reacted with a chromogenic substrate for 30 minutes at 37°C. The bioassay is based on the ability of toxic contaminants to inhibit *de novo* synthesis of inducible enzyme ß-galactosidase in a special bacterial strain of *E. coli*, which is highly sensitive to a wide spectrum of toxic substances. The bacterial response to toxic contaminant(s) is measured by the intensity of yellow colour developed. The higher the intensity the lower the toxic level is. Conversely, the most toxic level is represented by no yellow intensity.

The Microtox Solid-Phase testing procedure was used following the procedure described by Tung et al. 1991 and 1991a. The Microtox Reagent microorganisms are exposed directly to an aqueous suspension of the sample. After incubating for 20 minutes at room temperature, the organisms are separated with a filter column for analysis using 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 EC_{50}'s, 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 EC_{50} value > 19,000 ppm. A "CLEAN" or uncontaminated soil sample is defined as a reference soil which has an EC_{50} value at or above 2% using the Microtox Solid-Phase toxicity testing procedure (Tung et al. 1991).

Table 1. Toxicity data obtained from sediments using the DSTTP the Microtox solid-phase procedure.

SAMPLE #	DSTTP Sample concentration (%) required to inhibit 100% ß-galactosidase production	MICROTOX SOLID PHASE Sample concentration (%) required to produce EC_{50} 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

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 EC_{50} 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 EC_{50} 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. Dutton et al. (1988) noted that ß-galactosidase activity was inhibited only by heavy metals while ß-galactosidase biosynthesis was 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 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. Later chemical analysis confirmed that this incinerator ash had high level of metals. 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

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
Total assay time	3 hours	1 hour
Type of test	Semi-quantitative	Quantitative
Sensitivity	High	Moderate
Endpoint	ß-galactosidase inhibition	Luminescence inhibition
Labour	miminum	moderate

procedure tests a maximum sample concentration of 10%. This factor may help explain, in spite of testing for an

EC_{100} 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.

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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