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Comparative Assessment of Two Solid-Phase Toxicity Bioassays: The Direct Sediment Toxicity Testing Procedure (DSTTP) and the Microtox® Solid-Phase Test (SPT)

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Traditionally, the majority of environmental monitoring programs are based on chemical tests. Chemical analyses usually focus on selected "priority pollutants" and consequently neglect many other pollutants. Furthermore, chemical tests can not estimate synergistic effects among compounds in a mixture. However biological toxicity testing is based on the exposure of test organisms to all of the bioavailable chemicals in a sample and then noting changes in biological activity (Ribo et al., 1985).

Usually in estimating the toxicity of sediments, pore water (Giesy et al.,1987; Dutka et al., 1991) and/or solvent extracts (Hoke and Prater, 1980; Kwan et al., 1990; Dutka et al., 1991) are used to estimate the level of soluble or extractable bioavailable toxicants. However, it is often difficult to detect the total presence of the bioavailable toxicants due to the low concentration, low solubility and/or insolubility in the extracting solvents, incorrect extracting solvents and/or procedures (Kwan, 1993). Also, when organic solvents are involved, they must be diluted to their maximum allowable concentration (MAC) before the bioassays can be performed due to the toxicity effects of the solvents themselves (Kwan and Dutka, 1990). Direct sediment toxicity testing can circumvent these problems (Brouwer et al., 1990; Kwan and Dutka 1992a, b; Kwan, 1993). Direct sediment toxicity testing detects the total toxic response of soluble and insoluble, organic and inorganic, and volatile and non-volatile contaminants in the samples. Most importantly, direct sediment toxicity testing detects additive, synergistic and antagonistic effects and negates any solvent interferences. Thus direct sediment toxicity testing results indicate true bioavailability.

In this study we have compared the reproducibility and sensitivity of two newly developed solid-phase toxicity tests and their kits (Sediment-ChromoPad and Microtox® SPT) using 58 sediment samples. These sediments were collected from rivers, lakes and bays in Canada and USA: Toronto Harbour (11), Detroit River (15), Port Hope Harbour (4), St. Lawrence River (10), Cuyahoga River (4), Fox River (2), Lake Erie (2), Monroe Harbour (2), Rouge River (2), Sandusky Harbour (2), Toledo Harbour (1), Trenton Channel (3). Positive (toxic) and negative (non-toxic) controls were included in the screening procedures. The results of this study are presented and discussed.

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METHODS AND MATERIALS

Sediment samples were collected by various research organizations and universities and were submitted to the Priority Substances Project, Aquatic Ecosystem Protection Branch for solid-phase bioassay screening tests. The sediments were kept refrigerated at all times (melting ice in the field and at 2-4°C in the laboratory). The positive control sample was an incinerator ash which was received from the Hessische Industriemull GmbH (HIM) hazardous waste incinerator in Biebeshiem, Germany. The negative control sample was a natural clean sediment collected from Big Creek, Long Point, Lake Erie, Ontario. The chemical composition of the positive and negative controls are shown in Table 1.

Table 1. Chemical composition of Hessische Industriemull GmbH (HIM) incinerator waste and sediment sample collected from Big Creek, Long Point, Lake Erie, Ontario

METALS	INCINERATOR WASTE μg/gm	LAKE ERIE SEDIMENT μg/gm
Aluminum	4630	0.83
Antimony	524	11.00
Arsenic	2.7	<5.00
Barium	152	62.00
Boron	940	-
Cadmium	552	<0.20
Chromium	324	15.00
Copper	3878	14.00
Iron	11,780	1.40
Lead	5661	20.00
Magnesium	3389	10.30
Manganese	288	616.00
Mercury	116	-
Potassiu	14,950	62.00
Sodium	220,000	0.90
Strontium	-	117.00
Tin	1365	<20.00
Titanium	4678	3.20
Vanadium	<10	15.00
Zinc	10,072	62.00

This bioassay was performed following the procedure developed by Kwan, 1993 with some modifications, according to the manufacture instructions (Environmental Bio-detection Products Incorporation, 14 Abacus Road, Brampton, Ontario, Canada, L6T 5B7).

The Microtox® Solid-Phase Test was used following the procedure detailed in the Microtox® Manual, (1992) with the following modifications:

1. Before the test, a vial of previously frozen (-60°C) lyophilized bacteria (*Photobacterium phosphoreum*) was thawed in a 4°C refrigerator for one hour.
2. After thawing, the Reagent was prepared by mixing the lyophilized bacteria with 1 mL precooled (4°C) Microtox® Reconstitution Solution. The Reagent was mixed and stored in a clean cuvette and kept in the cooling well in the Microtox® Toxicity Meter for 30 min. before the Solid-Phase Test was performed.
3. After the 30 min. incubation, 700 μL Reagent was pipetted from the cooling well into a bottle of Microtox® Solid-Phase Diluent (50 mL) which had been precooled at 15°C. The bacteria-diluent mixture was thoroughly mixed by inverting the bottle several times.
4. The bacteria-diluent mixture was incubated at 15°C for 10 min.
5. After the 10 min. incubation, a repeater pipet was used to dispense the bacteria-diluent mixture into a set of 15 Solid-Phase Test tubes.
6. Then the rest of the steps were followed according to the Microtox Manual, (1992).

Kwan (1989) noted that when working with coloured samples the colour reduction was not a function of concentration, therefore the Microtox® Colour Correction Procedure was applied to all 58 test samples and controls to obtain the final toxicity readings (colour corrected data) rather than just making corrections on samples that had visible colour or turbidity at the EC_{50} concentration as recommended by Microbics. The following colour correction procedure was modified to maximize the sensitivity and at the same time to minimize the variations of the procedure from the Microtox® Solid-Phase Test.

1. The Microtox® Reagent was prepared by mixing 1 vial of lyophilized bacteria (4°C) with 1000 μL of Reconstitution Solution (4°C) and stored in a clean cuvette in the cooling well in the Microtox® Toxicity Meter for 30 min.
2. A sample dilution close to the EC_{50} value as shown in the Solid-Phase Test was prepared in a SPT tube and incubated at 15°C for 20 min.
3. After the incubation a Microtox Solid-Phase Filter Column was inserted into the SPT tube containing the sample dilution.
4. Then 1 mL of filtered sample dilution was used for colour correction procedure to obtain the I_t reading as instructed in the Microtox manual (1992). The sample dilution was mixed several times before replacing with 1 mL diluent to obtain the I_2 reading. This is to ensure that all precipitates sunk at the bottle of the cuvette was removed so as not to give any false reading in the final blank reading (I_2).

RESULTS AND DISCUSSION

Toxic (positive) and non-toxic (negative) control samples were used to monitor

the sensitivity and reproducibility of the Direct Sediment Toxicity Testing Procedure (DSTTP) and the Microtox® SPT. Table 2 presents the results of 10 replicate bioassays from both kits. The mean percent of sample required to produce the DSTTP EC_{100} and Microtox® SPT EC_{50} results were 6.25 and 0.07 respectively. Both tests indicated that the positive control sample was very toxic. The reproducibility of both tests with the positive control sample was excellent (DSTTP, S.D.=0.00 ; SPT, S.D.=0.01).

Table 2. Results of 10 replicate analyses of the positive (toxic) and negative control samples from the Sediment-ChromoPad and the Microtox® SPT. Results are reported as percentage of sample required to produce the EC_{100} and EC_{50} effects.

Trial #	Toxic sample		Non-toxic sample	
	DSTTP (EC_{100})	SPT (EC_{50})	DSTTP (EC_{100})	SPT (EC_{50})
1	6.25	0.07	>50	18.85
2	6.25	0.07	>50	13.52
3	6.25	0.06	>50	10.60
4	6.25	0.06	>50	14.12
5	6.25	0.09	>50	7.74
6	6.25	0.09	>50	8.50
7	6.25	0.07	>50	9.48
8	6.25	0.08	>50	11.92
9	6.25	0.06	>50	9.06
10	6.25	0.06	>50	18.51
$\overline{X}$	6.25	0.07	>50	12.23
SD	0.00	0.01	0.00	3.9

When performing the Microtox® SPT and the colour correction procedure on a sample, ideally identical samples and sample treatments should be used. However, it is rather difficult to duplicate an exact identical sample and sample treatments for two separate tests running at different times, due to the heterogeneity and complexity (physical and chemical properties) of the test samples , which are related to the proportion of the water content, the different grain sizes, the volume of organic matter, and the chemical composition of the sample. Each time a new sub-sample is prepared there are variations in all of the above. These variations are difficult to control and/or detected visually. However, the sensitive photomultiplier tube in the Microtox® Toxicity Meter can easily detect these variations which impact on the bacterial luminescence. As a result, a wide range of measurements (toxic levels) were observed in the negative control samples during the Microtox® Solid-Phase Test (Table 2). Conversely, the positive control sample which was a homogeneous incinerator ash with constant moisture, grain size, and chemical content, provided reproducible

results in both Direct Sediment Toxicity Testing Procedure (DSTTP) and Microtox® SPT.

Toxicity (EC_{100}) in the DSTTP is based on the complete inhibition of blue colour development on the chromogenic pad (Kwan, 1993). In this study, the sample toxicity was ranked according to the percent of sample which required to produce an EC_{100} effect: (a) very toxic, if the sample concentration was equal to or less than 12.5%; (b) moderate toxic, if the sample concentration was greater than 12.5% and less than or equal to 50%; (c) non toxic, if the sample concentration was greater than 50%. Data obtained from the Microtox® SPT were reported as percent of sample required to produce an EC_{50} effect (50% decrease in light output). Samples which produced EC_{50} values equal to or less than 0.5% were classified as very toxic, greater than 0.5% and equal to or less than 1.0% as moderate toxic; and greater than 1.0% as non toxic.

Table 3 presents the results of 58 sediment samples tested using the Direct Sediment Toxicity Testing Procedure (DSTTP) and the Microtox® SPT. Of the 58 sediment samples tested, 43 and 26 samples were detected toxicants using the DSTTP and the Microtox® SPT respectively. Of the 43 toxic samples detected using DSTTP, 9 were very toxic ($\leq$12.5% test sample volume) and 34 were moderately toxic (12.5% > 50% test sample volume). Similarly, among the 26 toxic samples detected toxicants using the Microtox® SPT, 10 were very toxic ($\leq$0.5%) and 16 were moderately toxic (> 0.5% $\leq$1.0%). Among the 58 sediment samples tested 24 sediment samples (TH-6,7,10,11; DR-14,16,17,18,22,24,25; SR-31,33,37,38,40; LE-41,42; CR-43,44; MH-50; SH-53,54; and TH55) were toxic to both bioassay tests. Although the range of responses in the two tests were recorded with different endpoints, the Spearman rank order correlation coefficient (r_s) indicated a positive correlation between the tests (r_s = 0.465; P $\leq$ 0.005).

When the 12 sampling sites were ranked based on the number of toxic samples detected per site, the ranking from the most toxic area to the least was as follows: Lake Erie, Cuyahoga River, Sandusky Harbour, Toledo Harbour, Detroit River, St. Lawrence River, Port Hope, Toronto Harbour, Rouge River, Trenton River, Monrose Harbour, and Fox River. Due to the lack of information on each site we were not able to confirm our findings. However, this study demonstrates that the two solid phase bioassays are in fact equally good for prioritizing sample sites/concern areas for future biological and /or chemical analyses.

Table 3. Results of 58 sediment samples tested by using the Sediment-ChromoPad and the Microtox® SPT. Results are reported as percentage of samples required to produce EC_{100} and EC_{50} effects.

SAMPLE #	DSTTP (EC_{100})	SPT (EC_{50})
TH01	50	27.57

Table 3. Cont'ed

SAMPLE #	DSTTP (EC_{100})	SPT (EC_{50})
TH02	>50	>100
TH03	50	>100
THO4	>50	>100
TH05	50	>100
TH06	25	0.09
TH07	6.25	0.83
TH08	>50	13.07
TH09	>50	6.27
TH10	6.25	0.35
TH11	12.5	0.50
DR12	50	9.00
DR13	50	41.00
DR14	50	0.73
DR15	>50	7.00
DR16	25	0.27
DR17	50	0.17
DR18	12.5	0.87
DR19	50	>100
DR20	50	3.89
DR21	25	5.91
DR22	50	0.58
DR23	50	5.10
DR24	12.5	0.73
DR25	12.5	0.39
DR26	25	1.99
PH27	>50	>100
PH28	<1.57	>100
PH29	25	13.3
PH30	25	15.2
SR31	50	0.88
SR32	>50	1.79
SR33	50	0.61
SR34	50	1.25
SR35	50	1.79
SR36	12.5	1.41
SR37	25	0.13
SR38	25	0.29
SR39	>50	1.38
SR40	50	1.00
LE41	25	0.76
LE42	12.5	0.18
CR43	50	0.78
CR44	50	0.86
CR45	50	0.50
CR46	50	2.75
FR47	>50	2.74
FR48	>50	5.92
MH49	>50	6.97
MH50	50	1.00
RR51	>50	2.71

Table 3. Cont'ed

SAMPLE #	DSTTP (EC_{100})	SPT (EC_{50})
RR52	50	1.12
SH 53	50	0.91
SH 54	50	0.76
TH55	25	1.00
TC56	>50	1.07
TC57	>50	0.72
TC58	>50	2.17

TH - Toronto Harbour; **DR** - Detroit River; **PH** - Port Hope Harbour; **SR** - St. Lawrence River; **CR** - Cuyahoga River; **FR** - Fox River; **LE** - Lake Erie; **MH** - Monrose Harbour; **RR** - Rouge River; **SH** - Sandusky Harbour; **TH** - Toledo Harbour; **TC** - Trenton Channel

Table 4 presents the summarized data of both tests. There were 43 and 26 samples detected toxicants using the Direct Sediment Toxicity Testing Procedure (DSTTP) and the Microtox® SPT respectively. The difference in the number of toxic samples detected between these two tests could have been due to (a) the different bacteria species used (Odum et al. 1979), (b) the sample contact time 120 min. (DSTTP) verses 20 min. (SPT) and the sample size 0.5 gm. (DSTTP) versus 0.3 gm (SPT) (Qureshi et al., 1984; Kwan, 1992), and (c) the variability of the Solid-Phase Test which is illustrated in Table 2. Finally, the difficulties of replicating test samples for the colour correction procedure may also result in either increasing or decreasing false-negative samples. Table 5 presents a brief summary comparison of the two procedures.

Table 4. Summary of the comparative toxicity studies results from 58 sediment samples using the Direct Sediment Toxicity Testing Procedure and the Microtox® Solid-Phase Test.

Toxicity Scoring	DSTTP	SPT
High	9	10
Moderate	34	16
Non-toxic	15	32

Table 5. Comparison of Direct Sediment Toxicity Testing Procedure (DSTTP) and the Microtox® SPT procedures

	DSTTP	MICROTOX® SPT
Kit form	yes	yes
Cost per kit	$100	$2652
# of assays per kit	4	40
Assay time from start to finish	4 hrs	1 hr
Sample through-put	high	high

Table 5. Cont'ed

	DSTTP	MICROTOX® SPT
Sample size	0.5 gm	0.3 gm
Laboratory space	minimum	moderate
Equipment	incubator (35°C) vortex mixer (optional)	incubator(15°C) Microtox®M500, computer & printer
Laboratory wares	disposable	disposable
Portability	portable	portable
Sensitivity	high	high
Reproducibility	high	moderate
Labour	low	moderate
Type of test	semi-quantitative	quantitative
Interferences	none	colour, turbidity
Pass/Fail test	yes	no

This study demonstrated that both Direct Sediment Toxicity Testing Procedure (DSTTP) and the Microtox® SPT are sensitive tests to detect bioavailable toxicants in solid phase samples. These solid phase tests are practical, reproducible, fast and relatively inexpensive compared to solid phase extraction procedures and have great potential for monitoring environmental solid samples and prioritizing sediment/soil sampling sites. These bioassays could provide, sewage treatment plant operators and oil drilling site operators, a quick means of assessing the toxicity of sewages and waste oil drilling muds prior to their disposable.

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