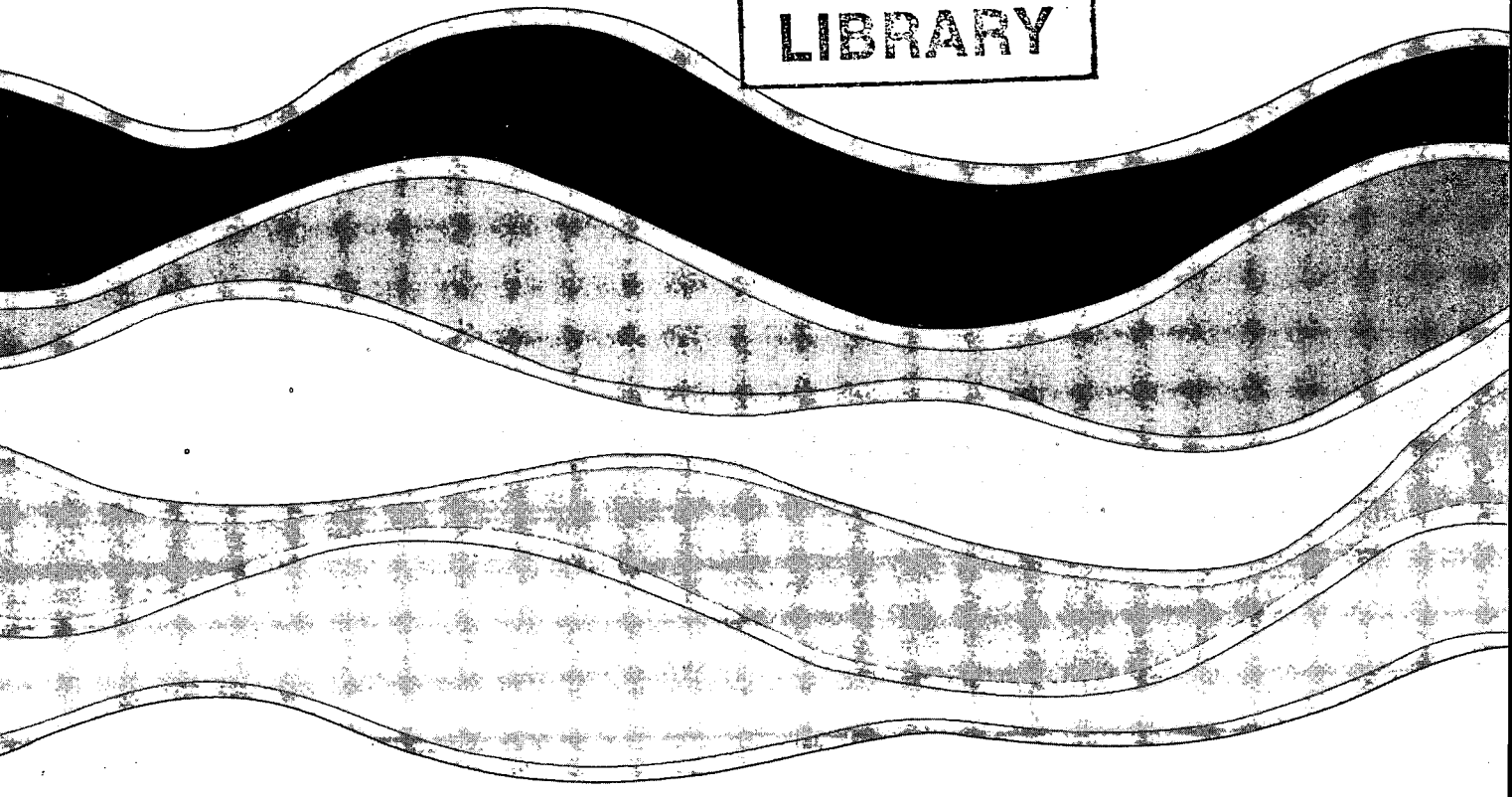
NATIONAL WATER RESEARCH INSTITUTE

INSTITUT NATIONAL de RECHERCHE sur les EAUX



A NOVEL BIOASSAY APPROACH: DIRECT APPLICATION OF TOXI-CHROMOTEST AND SOS-CHROMOTEST TO SEDIMENTS

K.K. Kwan and B.J. Dutka

NWRI CONTRIBUTION 90-161

RRB 90-82

A NOVEL BIOASSAY APPROACH: DIRECT APPLICATION OF TOXI-CHROMOTEST AND SOS-CHROMOTEST TO SEDIMENTS

by

K.K. Kwan and B.J. Dutka

Rivers Research Branch
National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario, L7R 4A6

DECEMBER 1990
NWRI CONTRIBUTION 90-161

MANAGEMENT PERSPECTIVE

Procedures for the extraction of sediments, suspended sediments or suspended particulates for toxicity and genotixicity studies are becoming increasingly more complicated, time consuming and expensive. The original concept of using short-term bioassays was to quickly, efficiently and inexpensively test water, sediments, and suspended sediments for toxicant/genotoxicant responses, in order that samples could be prioritized for the more costly chemical analyses. In this report we describe an extremely simple procedure, the direct sediment test procedure (DSTP) which can be used with the Toxi-chromotest and SOS-Chromotest to test sediments or suspended sediments/particulates for the presence of toxicants or genotoxicants.

It is believed the DSTP will now greatly motivate researchers and stimulate bioassay testing of sediments or suspended sediments/ particulates as the cost in time and dollars of extracting samples will now be significantly reduced.

PERSPECTIVE GESTION

Les techniques d'extraction qui s'emploient dans l'analyse des sédiments, tels quels ou en suspension et des particules en suspension pour l'étude de leurs propriétés génotoxiques, se font de plus en plus complexes, longues et coûteuses. À l'origine, les épreuves biologiques rapides devaient permettre d'évaluer de façon rapide, efficace et peu coûteuse les propriétés toxiques et génotoxiques de l'eau, des sédiments et des sédiments en suspension pour déterminer quels échantillons devraient passer en priorité aux analyses chimiques plus coûteuses. On décrit ici une méthode extrêmement simple, l'analyse directe des sédiments, qui peut servir avec le Toxi-Chromotest et le SOS-Chromotest à détecter la présence de produits toxiques ou génotoxiques dans les sédiments et dans les sédiments ou les particules en suspension.

On pense que l'analyse directe des sédiments motivera beaucoup les chercheurs et stimulera l'évaluation des sédiments, tels quels ou en suspension et des particules en suspension, car le temps et le coût d'extraction seront dorénavant réduits dans une mesure significative.

ABSTRACT

Routine testing of sediments or suspended sediments for toxicant or genotoxicants by the bioassay route often involves time consuming and expensive organic extraction procedures. In most instances these extraction procedures are more time consuming and costly than the bioassays which will be used on these extracts. A direct sediment test procedure (DSTP) was developed to alleviate this problem and thus return bioassays to one of their original roles i.e. short quick screening tests to identify priority samples for more intensive chemical analysis. The DSTP was developed in conjunction with the Toxi-Chromotest and SOS-Chromotest kits. The SOS-Chromotest can be used with or without S-9. Results presented in the paper indicate the sensitivity of DSTP and cost effectiveness compared to some commonly used sediment extraction procedures.

RÉSUMÉ

L'analyse de routine des sédiments, tels quels ou en suspension, pour détecter les toxiques et génotoxiques par des épreuves biologiques comporte souvent de longues et coûteuses opérations d'extraction au moyen de solvants organiques. Dans la plupart des cas, l'extraction demande plus de temps et revient plus cher que les épreuves biologiques qu'on fera subir aux extraits. On a mis au point une méthode d'analyse directe des sédiments pour réduire ces inconvénients et rendre ainsi aux épreuves biologiques l'un de leurs rôles originaux, soit celui d'épreuves de détection courtes et rapides permettant de déterminer quels échantillons doivent subir en priorité une analyse chimique plus poussée. L'analyse directe a été mise au point avec les nécessaires Toxi-Chromotest et SOS-Chromotest. Ce dernier peut s'utiliser avec ou sans le S-9. D'après les résultats présentés ici, au point de vue de la sensibilité et du rapport coût-efficacité, l'analyse directe est comparable à certaines des méthodes d'extraction couramment utilisées pour l'analyse des sédiments.

INTRODUCTION

The application of chronic and acute toxicity screening tests to environmental studies has been carried on for many years. However, during the last 10 - 15 year period, there has been a great emphasis on the development of acute toxicity screening tests using microorganisms or their properties as indicator systems (Liu and Dutka 1984, Bitton and Dutka 1986, and Dutka and Bitton 1986).

Two microbial colorimetric bioassays have been recently developed, SOS-Chromotest and Toxi-Chromotest (Quillardet et. al. 1982, Fish et. al. 1985, and Orgenics 1985, 1990), and marketed by Orgenics Ltd. (Israel) for the detection of genotoxic (SOS-Chromotest) and toxic activities in chemicals, pharmaceuticals, food stuffs, food additives, and cosmetics. The tests were later applied to environmental samples such as water, sewage and sediments (Xu et. al. 1987).

The SOS-Chromotest is based on the direct measurement of the damage to DNA through the actions of the SOS DNA repair system. In this test the visual monitoring of the results of genotoxic activity on bacterial cells is made possible by the SOS response to E. coli DNA damaging agents which results in a rapid biosynthesis of the enzyme beta-galactosidase. The enzyme's concentrations can be determined colorimetrically after the addition of ONPG (O-nitrophenyl-beta-D-galactopyranoside).

The Toxi-Chromotest is based on the ability of toxicant(s) to inhibit the de novo synthesis of an inducible enzyme (beta-galactosidase) in a specially mutated strain of E. coli (Orgenics 1985).

In the routine screening of sediments and suspended sediments by the various bioassay procedures, it is very difficult at times to detect the presence of toxicants and genotoxicants due to their very low concentrations. To circumvent this known problem,laboratories resort to a variety of concentration and extraction procedures. In our laboratory we extract the pore water from the sediment or suspended sediment and then add Milli-Q water to the de-watered sediment to remove any water soluble chemicals (Dutka et. al. 1990). Both of these extracts will be tested for the presence of toxicants and genotoxicants using a battery of bioassay procedures (Dutka 1988). This extracted sediment can then be treated in a variety of ways, alone or in combination, to extract the organic constituents which may have toxic or genotoxic activity. After extraction the extract is usually concentrated and exchanged if necessary into 1 mL of 100% DMSO (Dimethyl sulphoxide) and tested by a variety of bioassays at a 1% DMSO level. With other solvents such as methanol or combination of 10% methanol and 10% DMSO, the extracts may be tested at the 3% to 100% level (Unpublished data - Kwan) respectively depending on the bioassay being used. However, due to the toxic/genotoxic effects of the solvents themselves, the solvents must be diluted to their Maximum Allowable Concentration (MAC) (Kwan and Dutka 1990) which makes it difficult to detect the low levels of toxicant(s)/genotoxicant(s) in the sediment or suspended sediment samples.

To circumvent this problem we have developed a procedure by which sediments or suspended sediments can be tested directly without resorting to various extraction/concentration procedures. This direct sediment testing procedure (DSTP) has initially been adapted for use

with two bacterial bioassay kits, the SOS-Chromotest and the Toxi-Chromotest. This DSTP is based on the direct application of a specific volume of bacterial suspension to the freshly collected sediment or suspended sediment.

In this paper we will describe the DSTP and present bioassay data comparing DSTP to the solvent extracted sediments.

METHODS AND MATERIALS

Sample Collection

Five sediment samples were collected with an Ekman dredge from the Kaministiquia River, Thunder Bay, Ontario. These samples were placed into individual sterile plastic bags, iced and returned to the ecotoxicology laboratory for processing.

Two suspended sediment samples were collected from the Nith River in South-western Ontario by means of a model KDD 605 Westphalia continuous flow industrial centrifuge. At each sampling site approximately 2000 litres of Nith River water was centrifuged at a pumping velocity of 6 L per min. The suspended sediment was collected from the bowl, thoroughly mixed and then placed into a sterile bag, iced and returned to the laboratory for processing.

Sample Preparation - Sediment

From each of the five sediment samples, 50 grams of sediment were weighed and placed into an acid washed and Milli-Q water (Dutka et al. 1989) rinsed (5 times) BOD bottle to which 50 mL of Milli-Q water was added. The bottle was stoppered and vigorously shaken by hand for two minutes after which the entire contents were placed into a

centrifuge tube and centrifuged for 20 minutes at 10,000 rpm in a refrigerated centrifuge. The supernatant was collected and used in the toxicity/genotoxicity screening tests.

The above process was repeated with solvents, 10% methanol and 10% DMSO.

A total of 15 extracts (5-Milli-Q; 5-10% methanol and 5-10% DMSO) were produced.

Sample Preparation - Suspended Sediment

Due to the clarity of the Nith River, only a small amount of suspended sediment was collected from both sites, therefore for each extraction procedure, as per sediment samples above, only seven grams of suspended sediment could be used. Consequently, this resulted in a total of six extracts (2-Milli-Q, 2-10% methanol and 2-10% DMSO).

Toxi-Chromotest

The Toxi-Chromotest and SOS-Chromotest were used following the procedures described by Orgenics Ltd. (1985, 1990) with some modifications. These modifications involved direct contact between sediment/suspended sediment and bacterial suspension and chromogen incubation time. Details of the Toxi-Chromotest procedure are as follows:

1. Prepare the working bacterial suspension (E. coli) following the instructions provided in the kit;
2. place 0.5 gm of sediment into a sterile test tube containing 1.0 mL of the bacterial suspension;
3. mix the sediment and bacterial suspension in the test tube with a vortex mixer for 10 seconds;
4. incubate the mixture for 90 minutes at 35°C;

5. prior to the incubation period, prepare a microplate as in Figure 1;
6. dispense 200 uL of positive control standard (Hg^{++} 4ppm) into the first well of column 1 (i.e. well 1A) in the microplate;
7. into wells 1B through 1H dispense 100 uL of sample diluent, provided in the kit;
8. transfer 100 uL of Hg^{++} solution from well 1A to 1B, 1B to 1C, 1C to 1D, 1D to 1E, 1E to 1F, 1F to 1G, 1G to 1H and discard the last 100 uL from well 1H;
9. in column 2, dispense 100 uL of diluent into all wells, A to D;
10. the first four wells (2A to 2D) are used as machine blanks and the latter four wells (2E to 2H) will be the negative controls;
11. dispense 100 uL of bacterial suspension into all wells in columns 1 and 2, except the machine blank wells;
12. dispense 100 uL of reaction mixture, provided in the kit, into the machine blank wells;
13. incubate the microplate together with the bacterial-sediment tubes for 90 minutes at 35°C;
14. after the 90 minute incubation, pipet four 100 uL aliguots of sample (replicates) from the bacterial-sediment tube, into wells labelled corresponding to the tubes;
15. add 100 uL of yellow chromogenic substrate (prepared according to the instructions given in the kit) to all wells containing standard, blanks, controls and samples;
16. immediately after the addition of yellow chromogenic substrate, measure the optical density (O.D.) of each well at 405 um wavelength using the Vmax kinetic microplate reader (molecular Devices Corporation, Menlo Park California). These are the background readings at time zero;

17. incubate the microplate at 35°C for 90 minutes; and
18. after the 90 minute incubation, measure the O.D. of each well at 405 um wavelength. These are the final readings.

Toxicity Calculations

A sample is considered toxic if the mean optical density of the sample is less than the mean optical density of the control. Toxicity of a sample is expressed as percentage of inhibition. The percentage of inhibition is calculated from a comparison of the optical densities of the sample and the control.

$$\% \text{ of inhibition} = \frac{\bar{X} \text{ o.d.(c)} - \bar{X} \text{ o.d. (s)}}{\bar{X} \text{o.d(c)}} \times 100$$

where, $\bar{X}$ o.d.(c) = Mean Optical Density of Control

$\bar{X}$ o.d.(s) = Mean Optical Density of Sample

SOS-Chromotest

The SOS-Chromotest procedure with and without S-9 activation is similar to the Toxi-Chromotest procedure with the following exception, the Hg^{++} standard is replaced with 100 ppm 2-Amino-Anthracene (2AA) (with S-9), 10 ppm 4 Nitro quinoline oxide (without S-9). The contact time between sediment and the bacterial suspension is two hours. The reaction time for the sample, chromogen and the dry alkaline phosphatase substrate is 90 minutes. The S-9 mix when required is incorporated into the bacterial suspension and growth medium of the SOS-Chromotest. Details of the procedure are as follows:

1. Prepare overnight bacterial culture of E. coli, following the instructions provided in the kit;

2. prepare 10 mL fresh S-9 mix following the instructions provided in the kit;

3. prepare 10 mL growth medium and working bacterial suspensions for direct and indirect (s-9 activation) assays as follows;

	S-9 Mix	Bacterial Culture	Growth Medium
Direct assay	-	4.5 mL	5.5 mL
Indirect assay	2.5 mL	4.5 mL	3.0 mL
Growth with S-9 mix	2.5 mL	-	7.5 mL

4. prepare two sets of test tubes labelled from 1 to 8, one set for direct and the other for indirect assays;

5. dispense 2 mL of bacterial suspension into all test tubes labelled #1 and 1 mL of bacterial suspension into all test tubes labelled 2 to 8;

6. place 1 gm of sediment into tube #1 and mix the sediment and the bacterial suspension with a vortex mixer for 10 seconds;

7. transfer 1 mL of the mixture from the first tube into the 2nd, 2nd to 3rd, 3rd to 4th till the 8th tube and discard the last 1 mL from tube 8;

8. incubate the tubes for 2 hours at 35°C;

9. dispense 10 uL of standards and standard dilutions (4NQO and 2AA), blanks and controls (provided from the kit) into appropriated wells of the labelled microplate (Fig. 1);

10. dispense 100 uL of bacterial suspensions (with and without S-9) into wells containing standards and standard dilutions and controls blank wells;

11. dispense 100 uL of growth medium with and without S-9 mix, into appropriated machine blank wells;

12. incubate the microplate together with the bacteria-sediment tubes for 2 hours at 35°C;

13. after the 2 hour incubation, dispense four 100 uL of each sample and sample dilutions (tubes 1 to 8) from the bacteria-sediment tubes into the microplate wells labelled corresponding to the tubes;
14. add 100 uL of chromogen and dry alkaline phosphatase substrate (prepared according to the instructions given in the kit) to all wells containing standards, blanks, controls, sample and sample dilutions;
15. immediately after the addition of the chromogen, measure the optical density (O.D.) of each well at 405 and 620 um wavelengths using the Vmax kinetic microplate reader. These are the background readings at time zero;
16. incubate the microplate at 35°C for 90 minutes;
17. after the 90 minute incubation, measure the O.D. of each well at 405 and 620 um wavelengths. These are the final readings.

Genotoxicity Calculations

The genotoxicity of a sample is expressed by the Induction Factor. The Induction Factor value is calculated following the procedure described by Xu et. al. 1989. If the Induction Factor of a sample or a sample dilution is equal to or greater than 1.3 the sample or the sample dilution is considered genotoxic.

RESULTS AND DISCUSSION

Table 1 presents Toxi-Chromotest data obtained from five sediments and two suspended sediments using three solvent extraction procedures and the direct sediment testing procedure (DSTP). As in all instrumentation bioassay tests there is a background variable

response due to many factors, such as the number of test organisms, the health of each organism, growth phase of the organisms, diluting and pipetting variations, age of the chromogenic substrates, the uneven thickness of the microplate, scratches and finger prints on the microplate, etc. With the Toxi-Chromotest we tend to accept low responses, 10% and under as background noise and/or suspected toxicant activity. For higher values due to variations in bacterial populations, pipetting volumes, stresses, time differences etc. we tend to accept differences between two sets of results of up to 20% (e.g. % inhibition of 30 would be comparable to % inhibition of 24 - 36% inhibition), as providing equivalent results.

From Table 1 it can be seen that Milli-Q water extracts were completely negative for toxicant activity in four samples (#1, #5, #6 and #7) and for the other three samples, percentage inhibition is within the background noise level and may be considered doubtful or negative.

With the methanol extracts, the two suspended sediments were negative for toxic activity and the rest of the extracts indicate a low level presence of toxic activity with a maximum inhibition of 16.9%. DMSO extracts results were more variable than the methanol results with three non-toxic samples (#1, #6 and #7) and one sample (#2) producing a 25.8% inhibition, clearly a much higher response than any of the methanol extracts.

The DSTP data indicate that one suspended sediment (#6) which produced a non toxic response, produced a similar non toxic response with the other three extracts while sample #5 produced inhibition (9.5%) very similar to those of 10% methanol (10.5%) and 10% DMSO (11.9%). The remaining DSTP results were significantly greater

(greater inhibition) than those seen with the other extraction procedures. Highlighted in these observations would be sample #7 (suspended sediment) which was completely negative for toxicant activity in all three solvent extraction procedures, but with the DSTP, the greatest percent inhibition (65.8%) i.e. the greatest toxic effect, was found with this sample. Thus from Table 1 it can be seen that in five of the seven samples the DSTP produced the greatest toxicant activity and that 10% MeOH may have a slight selective superiority over 10% DMSO in these samples.

In Table 2, the results obtained from sediments and suspended sediments using the SOS-Chromotest kit and the three extracts (Milli-Q water, methanol and DMSO) and DSTP, are displayed. In this series of tests, no S-9 was added. While induction factors as low as 1.06 have been reported (Lan et al. 1991) indicating the presence of trace amounts of genotoxicants, it has been our philosophy not to consider induction factors below 1.25 as indicating the presence of genotoxic activity, unless there are toxicity interferences and the sample has to be diluted to such an extent that the induction factor is $\geq$ 1.25 and a decreasing dose response can be shown. Data obtained from DSTP are reported at 12.5% sample concentration; the first sample concentration at which no toxic reaction was noted.

In Table 2, it can be seen that, three Milli-Q water, four methanol, two DMSO and five DSTP treated samples are shown having genotoxic activity. DSTP produced the highest induction factor of this series (1.6) in sample 7. In three of the samples (1, 4 and 7) DSTP also had the highest induction factors (1.4, 1.5 and 1.6) while Milli-Q and DMSO extracts also produced the highest induction factor in two samples each.

In these samples (Table 2), of the four procedures used with the SOS-Chromotest kit, DSTP is the most efficient and cost effective means for testing for genotoxicants in sediments or suspended sediments. Ten percent methanol and Milli-Q water extracts were (in the above order) the next most efficient extracting procedures in these samples.

Table 3 presents genotoxicity data obtained from sediments/suspended sediments using the SOS-Chromotest kit with S-9 activation, DSTP and three extracts. One of the most striking features of Table 3 when compared to Table 2, is that all the DSTP treated samples were positive for genotoxic activity and the induction factors were in most instances 80 to over 100% higher than samples tested without S-9 activation. The S-9 mix contains enzymes that metabolize promutagenic substances and convert them to mutagens (Ames et. al. 1975). Thus these data are highly suggestive that the chemicals in these sediments/suspended sediments may be pro-genotoxic. With the other three extraction procedures, we noted that four Milli-Q water extracts, four DMSO extracts and five MeOH extracts with induction factors greater than 1.25. Of these three extraction procedures, 10% Methanol produced the highest induction factors in six samples, none of which were as high as those found in the DSTP samples.

Thus from these data, it would appear that DSTP is the most effective means of preparing sediment and suspended sediments to be used in conjunction with the SOS-Chromotest kit.

It should also be noted that of all the samples tested by the Toxi-chromotest and SOS-Chromotest kits, sample 7, a suspended sediment sample, yielded in all instances with DSTP the highest

toxicity and genotoxicity values. We believe this observation is strongly supportive of the concept that the majority of toxicants and genotoxicants are transported in riverine systems bound to suspended sediments and suspended particulates (Rao et. al. 1990).

In summation, the direct sediment testing procedure (DSTP) in conjunction with the Toxi-Chromotest and SOS-Chromotest has been shown to have great potential as a simple, quick, inexpensive means of testing sediments, suspended sediments and suspended particulates for the presence of toxicants and genotoxicants. Furthermore, it seems reasonable to assume this procedure will circumvent the exceedingly costly and time consuming procedures presently used in extracting contaminants from solid samples for use in various bioassay procedures. We believe the DSTP with the recently developed solid phase assay procedure for use with the Microtox test (Tung et. al. 1990; Brouwer et. al. 1990) will greatly motivate researchers and stimulate bioassay testing of sediments, suspended sediments and suspended particulates as the cost in time and dollars of extracting samples will now be significantly reduced.

REFERENCES

Ames, B.N., J. McCann and E. Yamasaki 1975. methods for detecting Careinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mut. Res. 31, 347-364.

Bitton, G. and B.J. Dutka 1986. Toxicity Testing using Microorganisms. Volume I, ed. G. Bitton and B.J. Dutka. CRC Press Inc. Boca Raton, Florida.

Brouwer, H., T.P. Murphy and L. McArdle 1990. A sediment-contact bioassay with Photobacterium phosphoreum. NWRI Contribution 90-11. National Water Research Institute, Burlington, Ontario Canada.

Dutka, B.J. and K.K. Kwan 1984. Studies on a synthetic activated sludge toxicity screening procedure with comparison to three microbial toxicity tests. Toxicity Screening Procedures Using Bacterial Systems, ed. D. Liu and B.J. Dutka. Marcel Dekker, New York, pp. 125-138.

Dutka, B.J. and G. Bitton 1986. Toxicity Testing using Microorganisms. Volume II, ed. B.J. Dutka and G. Bitton, CRC Press Inc. Boca Raton, Florida.

Dutka, B.J. 1988. Priority setting of hazards in waters and sediments by proposed ranking scheme and battery of tests approach. Ger Jour. Appl. Zool. 75: 303-316.

Dutka, B.J. (editor) 1989. Methods for Microbiological and Toxicological Analysis of Waters, Wastewaters and Sediments. Dept. of Environment, River Research Branch, National Water Research Institute, Canada Centre for Inland Waters, P.O. Box 5050, Burlington, Ontario, Canada.

Dutka, B.J., T. Tuominen, L. Churchland and K.K. Kwan 1989. Fraser river sediments and waters evaluated by the battery of screening test technique. Hydrobiologia 188/189: 301-315.

Dutka, B.J., K.K. Kwan, S.S. Rao, A. Jurkovic, R. McInnis, G.A. MacInnis, B. Brownlee and D. Liu 1990. Ecotoxicological study of waters sediment and suspended sediments in the Athabaska, Peace and Slave Rivers. NWRI Contribution 90-88A. NWRI, Burlington, Ontario, Canada.

Fish, F., I. Lampert, A. Halachmi, G. Riesenfeld and M. Herzberg, 1985. The SOS chromotest kit - a rapid method for the detection of genotoxicity. 2nd Intern. Symp. on Toxicity Testing using Bacteria. Banff.

Kwan, K.K. and B.J. Dutka 1990. Simple Two-Step Sediment Extraction Procedure for Use in Genotoxicity and Toxicity Bioassays. Toxicity Assessment: An International Journal Vol. 5, p. 395-404.

Kwan, K.K., B.J. Dutka, S.S. Rao and D. Liu 1990. Mutatox Test: A New Test for Monitoring Environmental Genotoxic Agents. Journal of Environmental Pollution 65, 323-332.

Lan, Q., M. Dickson and M. Alvarez 1991. Evidence of genotoxic substances in the Niagara River watershed. Environ. Toxicol. Wat. Qual. 6: (in press).

Liu, D. and B.J. Dutka 1984. Toxicity Screening Procedures using Bacterial Systems. ed. D. Liu and B.J. Dutka. Marcel Dekker, New York.

Orgenics Ltd. P.O. Box 360, Yavne 70650, Israel. The Toxi-Chromotest (Version 2) July, 1985.

Orgenics Ltd. P.O. Box 360, Yavne 70650, Israel. The SOS-Chromotest (Version 5.3) Oct., 1990.

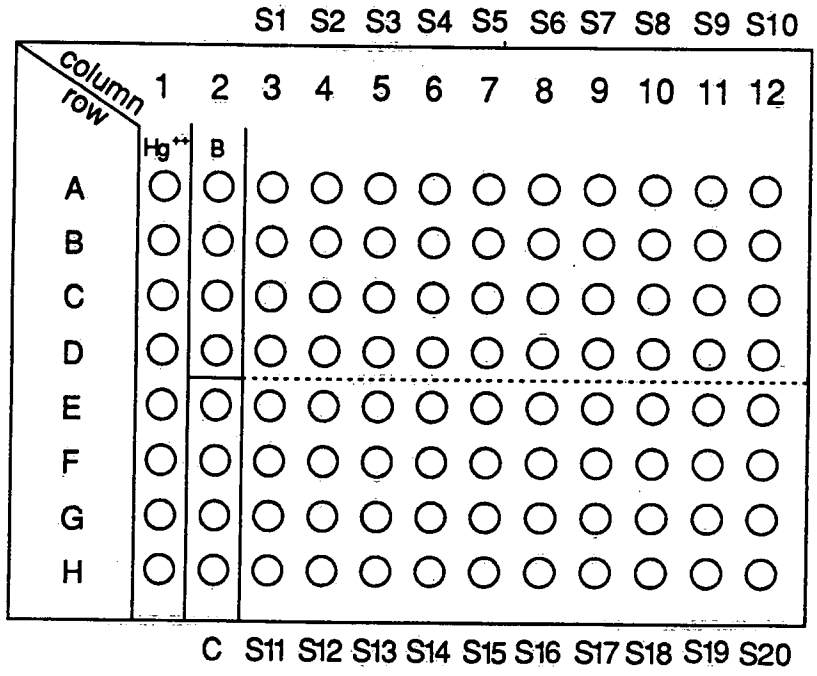
Quillardet, P., O. Huisman, R. D'Ari and M. Hofnung. 1982. SOS-Chromotest, a direct assay of induction of an SOS function in Escherichia coli K-12 to measure genotoxicity. Proc. Natl. Acad. Sci. USA, 79:5971-5975.

Rao, S.S., K.K. Kwan, B.J. Dutka, 1990. Toxicity Associated with fluvial suspended particulates. Ger. J. Appl. Zool. 78:4 (in press).

Tunq, K.K., M.G. Scheibner, C.C. Walbourn 1990. The solid phase assay: A new Microtox test procedure. Abstract. 17th Annual Aquatic Toxicity Workshop. Nov. 5-7 Vancouver B.C. Canada.

Xu, H., B.J. Dutka and K.K. Kwan 1987. Genotoxicity studies on sediments using a modified SOS chromotest Tox. Assess. 2: 79-87.

Xu, H., B.J. Dutka and K. Schurr. 1989. Microtitration SOS-Chromotest: A New Approach in Genotoxicity Testing. Toxicity Assessment: An International Journal Vol. 4, p. 105-114.

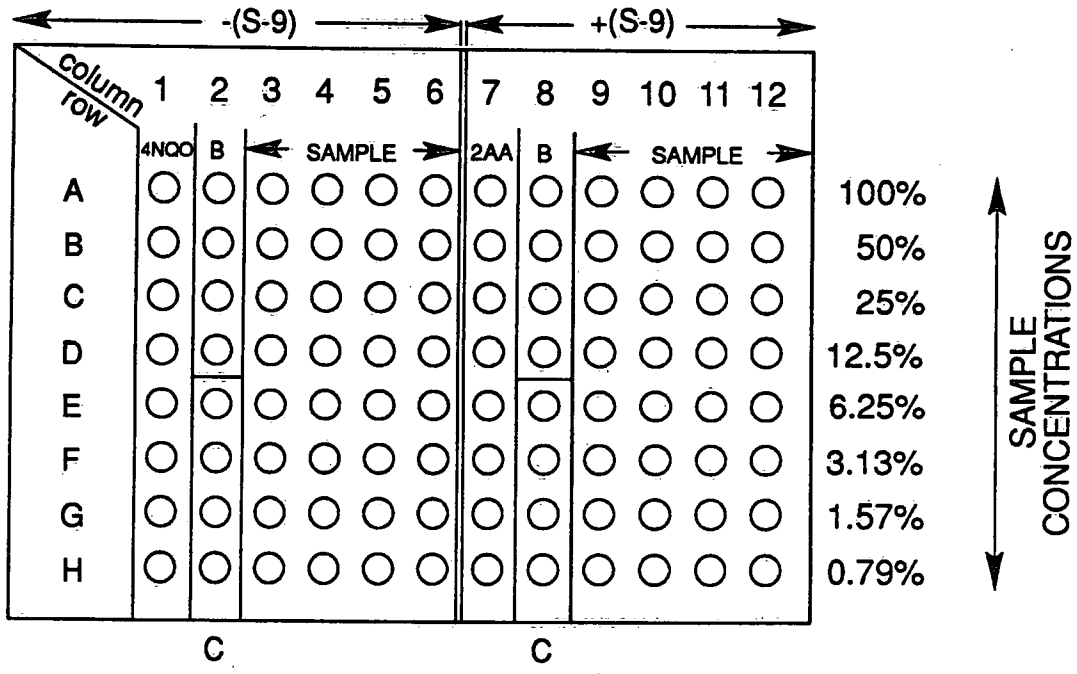


TOXIC-CHROMOTEST

B = Blank

C = Control

S = Sample



SOS-CHROMOTEST

Figure 1. Toxic-Chromotest and SOS-Chromotest Sample layout scheme in a 96 well microplate

Table 1. Toxicity data[1] obtained from sediment/suspended sediments using the Toxi-Chromotest kit, direct sediment testing procedure (DSTP) and three extracts. Toxicity values are expressed as percentage of inhibition of beta-galactosidase production.

	EXTRACTS			
SAMPLE #	Milli-Q H_2O	10% MeOH	10% DMSO	DSTP
1	0%	11.9%	2.7%	36.1%
2	3.0%	15.8%	25.8%	35.0%
3	3.3%	16.9%	15.2%	29.3%
4	6.7%	16.6%	14.7%	39.5%
5	0%	10.5%	11.9%	9.5%
6*	0%	2.8%	0%	2.5%
7*	0%	0%	0%	65.8%

* Suspended sediment

1 All results are based on four replicates

Table 2. Genotoxicity data[1] obtained from sediment/suspended sediments using the SOS-Chromotest kit without S-9, direct sediment testing procedure (DSTP) and three extracts. Genotoxicity is expressed as Induction Factors.

	EXTRACTS			
SAMPLE #	Milli-Q H_2O	10% MeOH	10% DMSO	DSTP
1	1.1	1.0	1.0	1.4
2	1.4	1.3	1.5	1.4
3	1.5	1.4	1.1	1.2
4	1.0	1.3	1.1	1.5
5	1.4	1.3	1.4	1.3
6*	1.1	1.0	1.0	1.1
7*	1.0	1.0	1.0	1.6

* Suspended sediment

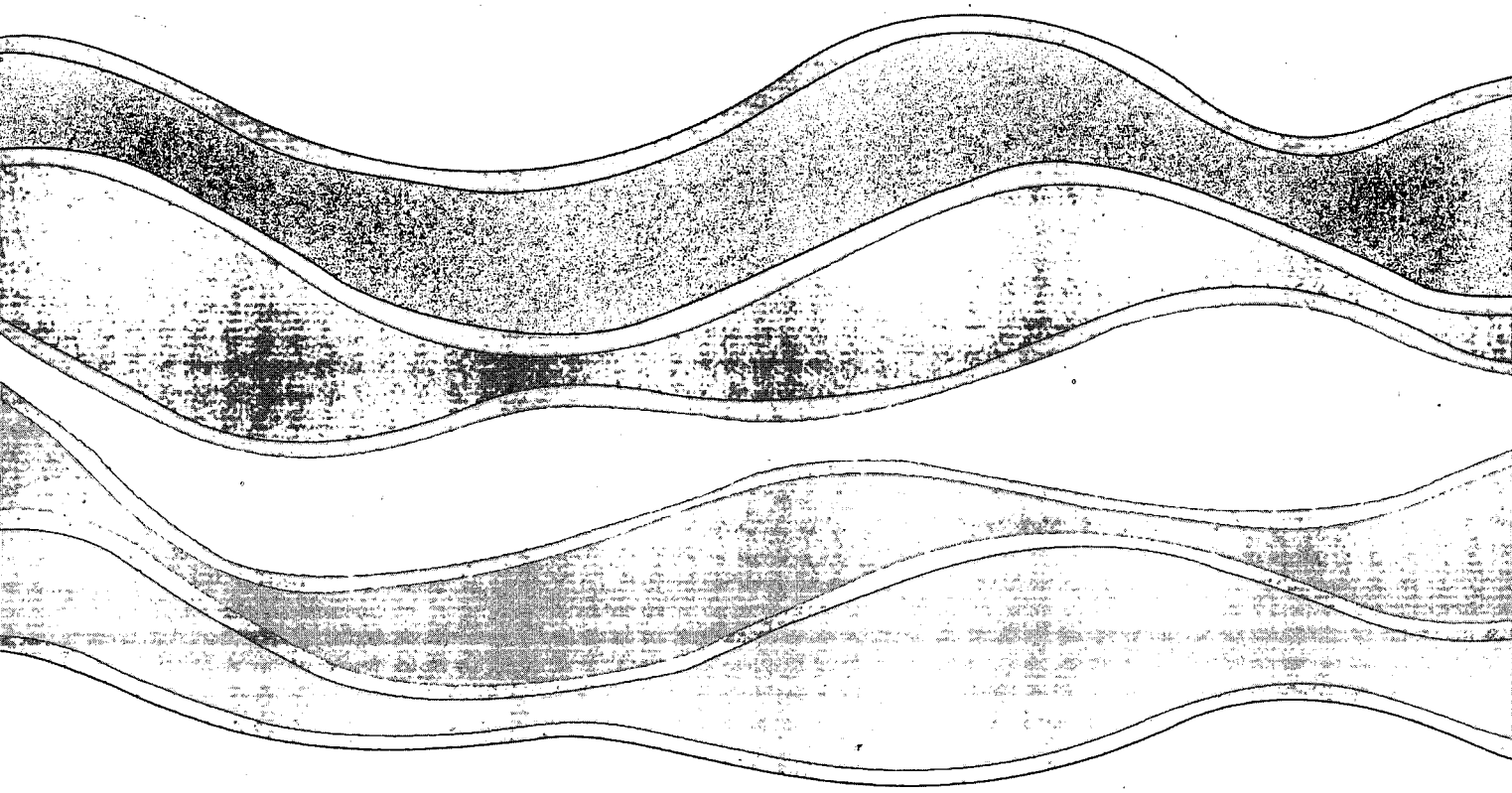
[1] All results are based on four replicates

Table 3. Genotoxicity data[1] obtained from sediment/suspended sediments using the SOS-Chromotest kit with S-9, direct sediment testing procedure (DSTP) and three extracts. Genotoxicity is expressed as Induction Factor.

	EXTRACTS			
SAMPLE #	Milli-Q H_2O	10% MeOH	10% DMSO	DSTP
1	1.3	1.6	1.3	2.8
2	1.2	1.4	1.4	1.8
3	1.3	1.3	1.0	2.8
4	1.0	1.1	1.0	2.8
5	1.3	1.3	1.6	2.2
6*	1.0	1.2	1.2	2.5
7*	1.9	2.0	1.6	3.0

* Suspended sediment

[1] All results are based on four replicates



NATIONAL WATER RESEARCH INSTITUTE
P.O. BOX 5050, BURLINGTON, ONTARIO L7R 4A6

Environment Canada Environnement Canada

Canada

INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX
C.P. 5050, BURLINGTON (ONTARIO) L7R 4A6