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NWRI CONTRIBUTION NO. 95-53

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MANAGEMENT PERSPECTIVE

Toxicity and genotoxicity screening of sediments, suspended sediments, suspended particulates, soils, solid wastes and other solid phase samples have provided monitoring agencies many difficulties and challenges over the years. Initially very few solid phase bioassays were performed because of technical difficulties. As microbial and enzyme based bioassays were developed or their potentials realized, solid phase extracts (water and solvent) were used to assess the toxicity of these solid phase samples. However, in the routine toxicity screening of solid phase samples, it was often difficult to selectively detect the presence of toxicants and genotoxicants due to their low concentration, low solubility or insolubility in the extracting solvents. Another important problem which was encountered especially with genotoxicants was the masking of any genotoxic activity by the activity of the toxicant. Even after solvent extraction and dilution procedures the genotoxicant activity was frequently not seen due to the dilution required to minimize or exclude the toxicants effect.

To address this problem laboratories resort to a variety of extracting solvents and concentration procedures. The use of solvent extraction on solid phase samples has often been very chemical specific and the procedures questioned as to what degree the samples have been changed during the extraction process. Also it has been noted that although sediments may contain high concentrations of toxic chemicals, toxicity or increased toxicity to organisms living in the sediments may not be observed. The bioavailability of toxic compounds to benthic organisms depends on the trophic position of an organism and any toxic effect to the organism depends on its relative sensitivity to interstitial and particle bound chemicals. Extraction/concentration procedures do impact on the original bioavailability of the chemicals in the sample and in doing so the potential toxicity can be estimated but the real *in situ* toxicity/genotoxicity is rarely known. Therefore, success in detecting the true toxicity of solid phase samples is still very limited.

This study describes a new SOS-Chromotest pad procedure for testing of sediment samples for genotoxicity and cytotoxicity, directly without extraction. Preliminary data show that

the bioassay is highly sensitive to direct-acting mutagens and can discriminate between genotoxic and cytotoxic pollutants. The bioassay procedure is easy to perform, requires minimal accessory equipment and is cost effective.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Le dépistage des substances toxiques et génotoxiques présentes dans les échantillons de sédiments, de sédiments en suspension, de particules en suspension, de sols, de déchets solides et autres matières en phase solide a posé nombre de difficultés et de défis aux organismes de surveillance au cours des années. Au début, on effectuait très peu de bioessais en phase solide à cause des difficultés techniques que cela présentait. À mesure que des bioessais à l'aide de micro-organismes et d'enzymes ont été mis au point et que les possibilités de ces bioessais ont été exploitées, on en est venu à utiliser des extraits en phase solide (eau et solvant) afin de déterminer la toxicité des échantillons en phase solide. Cependant, dans le dépistage habituel de la toxicité de ces échantillons, il était souvent difficile de distinguer la présence des substances toxiques et celle des substances génotoxiques, étant donné la faible concentration de ces substances et le fait qu'elles sont peu solubles, sinon insolubles, dans les solvants d'extraction. Un autre important problème rencontré, en particulier dans le dépistage des substances génotoxiques, résidait dans le fait que l'activité génotoxique était masquée par l'activité toxique. Même après l'application des techniques d'extraction au solvant et de dilution, il était fréquent que l'on ne puisse observer l'activité génotoxique à cause de la dilution nécessaire pour réduire ou empêcher l'effet des substances toxiques.

Afin de résoudre ce problème, les laboratoires ont recours à toute une variété de solvants d'extraction et de techniques de concentration. L'usage de solvants d'extraction sur des échantillons en phase solide s'est souvent appliqué à une substance chimique donnée, et les techniques utilisées ont été mises en cause parce que l'on s'interrogeait sur le degré de modification subi par les échantillons au cours du processus d'extraction. Même dans le cas de sédiments à teneur élevée en produits chimiques toxiques, signalait-on, il était possible qu'on ne réussisse pas à observer de toxicité ou de toxicité accrue vis-à-vis des organismes vivants contenus dans les sédiments. La biodisponibilité des composés toxiques pour les organismes benthiques est fonction de la position des organismes au sein du réseau trophique, et l'effet toxique pour un organisme donné varie selon la sensibilité de ce dernier à l'égard des substances chimiques interstitielles et des substances chimiques liées aux particules. Les techniques

d'extraction et de concentration ont effectivement une incidence sur la biodisponibilité initiale des substances chimiques présentes dans un échantillon, c'est pourquoi la toxicité-génotoxicité réelle sur le terrain est rarement connue, bien que l'on puisse en évaluer la toxicité potentielle. Ainsi n'arrive-t-on que dans une mesure très limitée à déterminer la toxicité réelle des échantillons en phase solide.

L'étude décrit une nouvelle technique de bioessai SOS-Chromotest effectué à l'aide d'un tampon, qui permet le dépistage direct, c'est-à-dire sans extraction, de la génotoxicité et de la cytotoxicité des échantillons de sédiments. Les premières données révèlent que ce bioessai est très sensible aux mutagènes à action directe et qu'il peut distinguer les polluants génotoxiques des polluants cytotoxiques. Il s'agit d'un bioessai facile à réaliser, qui n'exige qu'un minimum de matériel accessoire et qui est économique.

ABSTRACT

A modified SOS-Chromotest bioassay using a chromogenic pad (pad procedure) was developed to test for genotoxicity in sediments directly without extraction. This test is based on the de novo synthesis of ß-galactosidase enzyme by a genetically-engineered *E. coli* strain PQ37. In the bioassay, an exponential growth phase antibiotic-containing culture of the test bacterium is introduced into a series of tubes with the first tube containing 0.1 gram of sediment. Serial dilutions are then made and the tubes of sediment plus bacterial culture are incubated at 37°C for four hours, followed by placing a drop of each mixture on a chromogenic pad and additional incubation for 20 hours at 37°C. The solid particulates are then washed off with tap water and positive (genotoxic) activity is noted by the presence of a distinctive blue colour on the pad. The SOS-Chromotest pad procedure may be best used as a relative measure of genotoxicity by comparing results to a reference sample. In addition it can also determine sediment cytotoxicity by comparing samples spiked with a genotoxic standard (i.e., 4-nitroquinoline-N-oxide). Preliminary results suggest that this new bioassay is highly sensitive, consistent and discriminating.

RÉSUMÉ

Un bioessai SOS-Chromotest modifié à un tampon chromogène (technique du tampon) a été mis au point dans le but de permettre un dépistage direct, sans extraction, des substances génotoxiques présentes dans les sédiments. Cet essai repose sur la synthèse *de novo* de l'enzyme ß-galactosidase par une souche PQ37 d'*E. coli* mise au point par génie génétique. Dans ce bioessai, une culture de la bactérie d'essai contenant des antibiotiques en phase de croissance exponentielle est introduite dans une série de tubes dont le premier contient 0,1 gramme de sédiment. Des dilutions en série sont alors effectuées, et les tubes de sédiments ainsi que la culture bactérienne sont mis en incubation à 37 °C pour 4 heures. Une goutte de chaque mélange est ensuite déposée sur un tampon chromogène, et l'incubation se poursuit à 37 °C pendant encore 20 heures. Les particules solides sont alors éliminées avec de l'eau du robinet, et l'activité positive (génotoxicité) est révélée par l'apparition d'une teinte bleue distinctive sur le tampon. La technique du SOS-Chromotest à tampon convient surtout pour obtenir une mesure relative de la génotoxicité par comparaison des résultats avec ceux d'un échantillon témoin. De plus, cette technique permet de déterminer la cytotoxicité d'un sédiment par rapport à des échantillons auxquels on a fait un ajout connu d'une substance génotoxique standard (4-nitroquinoléine-N-oxyde). Les premiers résultats obtenus indiquent que ce nouveau bioessai est très sensible, constant et discriminant.

INTRODUCTION

Toxicity and genotoxicity screening of sediments, suspended sediments, suspended particulates, soils, solid wastes and other solid phase samples have provided monitoring agencies many difficulties and challenges over the years. Initially very few solid phase bioassays were performed because of technical difficulties. These bioassays were usually carried out by using higher organisms (earthworms, benthic invertebrates such as chironomids, mayflies, amphipods and fresh water oligochaetes) and seeds or plants which are normally found in some solid phase materials (Day et al., 1995). As microbial and enzyme based bioassays were developed or their potentials realized, solid phase extracts (water and solvent) were used to assess the toxicity of these solid phase samples. However, in the routine toxicity screening of solid phase samples, it was often difficult to selectively detect the presence of toxicants and genotoxicants due to their low concentration, low solubility or insolubility in the extracting solvents (Atkinson et al., 1985; Schiewe et al., 1985). Another important problem which was encountered especially with genotoxicants was the masking of any genotoxic activity by the activity of the toxicant. Even after solvent extraction and dilution procedures the genotoxicant activity was frequently not seen due to the dilution required to minimize or exclude the toxicant effects.

To address this conundrum laboratories resort to a variety of extracting solvents and concentration procedures. The use of solvent extraction on solid phase samples has often been very chemical specific and the procedures questioned as to what degree the samples have been changed during the extraction process. Also it has been noted that although sediments may contain high concentrations of toxic chemicals, toxicity or increased toxicity to organisms living in the sediments may not be observed (True and Hayward, 1990). The bioavailability of toxic compounds to benthic organisms depends on the trophic position of an organism and any toxic effect to the organism depends on its relative sensitivity to interstitial and particle bound chemicals (Swartz et al., 1986). Extraction/concentration procedures do impact on the original bioavailability of the chemicals in the sample and in doing so the potential toxicity can be estimated but the real *in situ* toxicity/genotoxicity is rarely known. Therefore, success in detecting

the true toxicity of solid phase samples is still very limited.

Over the past five or six years there has been a partial solution to the above problems. Qualitative and semi-quantitative direct solid phase toxicity testing procedures have been developed using bacterial systems (Dutka and Gorrie, 1989, Brouwer et al., 1990; Kwan, 1991) and using the immunochemical detection of a cell surface reporter protein of *Escherichia coli* (Stubner et al., 1994).

A sensitive, rapid, cost-effective and particularly simple genotoxicity bioassay is the SOS-Chromotest (Fish et al., 1987; Quillardet *et al.*, 1982). This test is based on the de novo synthesis of ß-galactosidase enzyme by a genetically-engineered *E. coli* (*strain PQ37*). As reported by Quillardet and Hofnung (1985) the tester strain used in the SOS-Chromotest carries a *sfiA::lacZ* fusion and has a deletion of the normal *lac* region so that β-galactosidase activity is strictly dependent on *sfiA* expression. In addition the strain is made genetically more susceptible to genotoxic agents: it is devoid of the excision repair pathway (*uvrA* mutation) so that a number of lesions are not, or are slowly processed, and it has a mutation (*rfa*) which renders the cell envelope more permeable to a number of compounds. Thus the triggering of the SOS response system can be used as a general and early sign of DNA damage. Over the past decade, the SOS-Chromotest has been shown to correlate well with the traditional Ames test in a wide variety of compounds (Quillardet and Hofnung, 1993). In addition, it is considerably more simple to use and cost-effective than most alternative genotoxicity bioassays available.

In an earlier study, a direct sediment testing procedure (DSTP) for genotoxicity was developed using the SOS-Chromotest microplate (Kwan and Dutka, 1992). However subsequent evaluation trials revealed that physical interferences were associated with the solid particulates and which could mask the detection of potentially genotoxic samples. The aim of this study was to circumvent these problems by replacing the microplate with a chromogenic pad.

METHODS AND MATERIALS

Bacteria and Reagents

Lyophilized cells of genetically-engineered *E. coli* strain PQ37 (Quillardet *et al.,* 1982), a component of the SOS-Chromotest™ kit (EBPI, Brampton, Ontario), were used as the bioassay test organism. The growth-medium component of the SOS-Chromotest™ kit was used for bacterial culturing. The β-galactosidase enzymatic reaction was determined by the chromogenic pad of the Sediment-ChromoPad™ kit (EBPI, Brampton, Ontario).

Genotoxic Standard Control

The direct-acting genotoxic compound 4-nitroquinoline-N-oxide (4NQO, SIGMA) was used as: (a) the positive control in solution without sediment, (b) with a 'clean' (reference) sediment, and (c) with test sediment samples. In spiked sediments, the 4NQO concentration was adjusted to 0.5 μg/mL in the suspension.

Sediment Samples

Fresh-water sediments collected for other ongoing projects were used to evaluate the new bioassay procedure. The reference sediment was collected from the Long Point area of Lake Erie, Ontario, and had been thoroughly washed with ultra pure water for use as a non-toxic control in the semi-quantitative direct solid-phase toxicity testing procedure (DSTTP; Kwan, 1993). Samples collected from the Temuco area and the homelands of the Chol Chol and Maquehue Mapuche in Chile, and samples collected from the River Elbe in Germany, were used in the evaluation study.

Preparation of Bacterial Culture

The day before the bioassay a vial of SOS-Chromotest kit's growth-medium was added to a vial of lyophilized bacteria (*E. coli* PQ37). The bacterial suspension was incubated for 16-18 hr at 37°C, then diluted in fresh growth-medium to give an optical density of 0.07 at 620 nm. The bacterial suspension was then supplemented with filter-sterilized ampicillin to a final concentration of 20 μg/ml.

Preparation of Sample and Bacteria Suspension and Sample Dilution in test tubes.

For each sample, aliquots of 0.5 mL bacterial suspension were added to 10 tubes (numbered 2 to 11). Aliquots of 1 mL bacterial suspension were added to the other test tubes (labelled 1 and Control). One hundred milligrams of sediment (fresh weight) was added to tube #1, then mixed thoroughly, after which 0.5 ml was removed and placed in tube #2, etc., for serial two-fold dilutions in tubes numbered 1 to 11 (representing sediment concentrations of 10% down to 0.01%, w/v). No sediment was added to the control tube. In addition, 4NQO-spiked dilutions were prepared. For each sample, another similar set of 12 test tubes was made up, but supplemented with 4NQO at a concentration adjusted to 0.5 μg/mL suspension in every test tube. Hence, a total of 24 suspensions in test tubes were prepared for each sample.

Incubation of Test Tubes

All tubes were incubated for 4 hr at 37°C.

Placement of Sediment and Bacteria Slurry on Pads

After incubation, each tube was thoroughly mixed and a drop of the slurry (ca. 20 μL) was placed on a chromogenic pad. The spots were positioned on the pad counter clockwise in decreasing concentrations. Five to six drops could be placed on a single pad. An additional drop of the negative control (no sediment added) was placed on the centre of each pad. All pads were incubated at 37°C for 20 hr, unless mentioned otherwise.

Data Recording and Analysis

After incubation, solid particulates were washed off the pad with tap water using a squirt bottle. The colour of each transfer spot was then visually observed. Based on the intensity of the blue colour developed in the positive control, a point rating scheme was used in which the most intense blue colour (indicative of a strong genotoxic effect) was given a colour index value of 5, while no blue colour was given a colour index value of 0 (Figure 1).

For each sample, either raw or 4NQO-spiked, eleven colour index values were recorded, corresponding to each of the 2-fold dilutions of sample material. The colour index

values, for each test sample, were combined in a Colour Index Profile (CIP), which is an 11-digit number representing (left to right) the colour index of the lowest dilution (or highest sample concentration) to the highest dilution (or lowest sample concentration). An example of CIP recording is illustrated in Figure 1.

Genotoxicity was determined by comparing the CIP of a test sample with the CIP of a reference sample. The reference sample was relatively free of toxic contamination, but otherwise of similar characteristics to the other test samples. Cytotoxicity was determined based on comparing the CIP of a test sample spiked with 4NQO, with the CIP of a 4NQO-spiked reference sample.

The first step in determining genotoxicity was to subtract the digit values of the reference-sample CIP digits from the corresponding CIP digit values of the test sample, resulting in an 11-digit net-CIP number. In the second step, all digits that were ≥ 2 of the net-CIP were added up, giving a numerical genotoxicity value. The genotoxicity value was used as quantitative measure to compare the genotoxic response between samples.

Cytotoxicity was determined similarly, but is based on the CIP of 4NQO-spiked material. First, the digit values of the sample 4NQO-spiked CIP were subtracted from the corresponding CIP values of the 4NQO-spiked reference sample. In the second step, all digits that were ≥ 2 of the net 4NQO-spiked CIP were added up, giving a numerical cytotoxicity value. The cytotoxicity value was used as a quantitative measure to compare cytotoxic response between samples.

RESULTS

Preliminary studies indicated the need to suppress bacterial background populations in the samples being tested, and this led to the augmentation of antibiotic (ampicillin) to the bacterial growth medium. During these preliminary studies a variety of contact periods (1.5 to 6 hr) between the *E. coli* bacteria and sediment were evaluated with the four hour contact being

selected as the optimal contact period (data not shown).

Along with this contact-period investigation, contact between the stressed cells and the indicator system (chromogenic substrate) was also being evaluated. Very soon after this research started it was realized that the chromogen system used in the SOS-Chromotest microplate kit could not be used with environmental sediments because of background colour interferences. However the recent development of the chromogenic pad (the Sediment-ChromoPad™ kit, EBPI, Brampton, Ontario) provided a possible solution to this problem.

The new SOS-Chromotest pad procedure responded well to pure solutions of 4-Nitroquinoline-N-Oxide (4NQO). Table 1 presents six sets of data collected in experiments repeated over a three week period. The table shows that in a short incubation period (i.e., 4 hr in test tubes plus 3 hr on pads) the bioassay would detect a genotoxic response to 4NQO at concentrations between 19.5 to 1,250 ng/mL, with maximal reaction (i.e., colour index of 4 - 5) occurring at 4NQO concentrations between 312.5 - 625 ng/mL. With a longer incubation period (4 hr in test tubes plus 20 hr on pads) the detection limit dropped to 9.8 ng/mL in all of the six repeat experiments, while the range concentrations with maximal reaction widened to 39 - 625 ng/mL.

The ability of the SOS-Chromotest pad procedure to discriminate between polluted and non-polluted sediment was demonstrated using a reference sediment sample, with and without 4NQO. Table 2 presents five sets of repeat experiments. The table shows that 4NQO at a concentration of 0.5 μg/mL was detected when sediment concentrations in suspensions were as high as 5% - 10% (w/v). However, maximal reaction (i.e., colour index of 4 - 5) was expressed at lower sediment concentrations (0.16% - 2.5%). The genotoxicity value, indicative of the level of genotoxic reaction of the 4NQO addition to the reference sediment was determined by comparing the bioassay reactions between the 4NQO-spiked sediment to the non-spiked sediment. The Genotoxicity Values obtained ranged between 34 to 48 (av. 40.0, S.D. 5.2)

In further evaluation of the bioassay, two environmental samples, BD and BE, were

tested, either non-spiked or spiked with 4NQO, by comparison with the reference sediment, in four repeat experiments. The results (Table 3) show no evidence of genotoxicity in these samples. On the other hand, cytotoxicity was noticed in both samples, based on the interference to the 4NQO-induced genotoxic reaction. Sample BE was toxic, under the bioassay conditions, at a concentration as low as 0.08%. The lowest concentration of sample BD that was toxic was 1.25%.

Further evaluation of environmental sediments is summarized in Table 4. The table shows a strong indication of genotoxicity in sample Elbe #27, over a wide range of sample concentration, from 5% to 0.31%. Only one other sample, Chile #12, gave a possible genotoxic response, but only at weak level (colour index = 2) and only at a single sample concentration (0.31%).

Cytotoxicity was expressed by sample Elbe #48, Elbe #13, Elbe #32, Elbe #27, Chile #12 and Elbe #45. The minimal toxic (sediment) concentrations of these samples that were toxic were 0.16%, 0.31%, 2.5%, 2.5%, 5% and 10%, respectively.

DISCUSSION

This study describes a modified SOS-Chromotest bioassay (pad procedure), used to detect the presence of bioavailable genotoxic and/or cytotoxic compounds in sediments directly without extraction. The bioassay is based on a genetically-engineered strain *E. coli* PQ37 and the SOS-Chromotest in a test tube (Quillardet *et al.*, 1982; Quillardet and Hofnung, 1993) or in a microplate (Fish *et al.*, 1987). The bioassay procedure described in this study utilized a chromogenic pad in order to enable the bioassay reaction to develop in direct exposure to solid particles. The bioassay response to genotoxic assault is measured by formation of distinctive blue colour on the pad. The strength of genotoxicity is measured by the intensity of colour developed on the pad. The level of genotoxicity in the sample is also measured by the range of sample concentrations (in a series of 2-fold dilutions) that induce detectable changes of colour.

The SOS-Chromotest pad procedure was developed for the purpose of screening large volumes of environmental samples. Key criteria of a practical bioassay for screening purposes include the following:

- sensitivity of detection of low-concentration toxic/genotoxic agents;
- ability to discriminate between toxic/genotoxic pollutants and non-toxic/non-genotoxic background;
- consistency of performance in repeat experiments;
- simplicity of performance; and
- cost effectiveness.

Sensitivity:

Sensitivity of detecting low-concentrations of a direct-acting genotoxic agent, 4-Nitroquinoline-N-Oxide (4NQO), was demonstrated in this study. The SOS-Chromotest pad procedure was able to detect a concentration of 4NQO as low as 9.8 ng/mL water solution (Table 1). By comparison, the sensitivity levels (minimum active concentrations) of 4NQO in other common screening bioassays are 39.2, 89.2, 11.8 and 1.2 ng/mL by the standard SOS-Chromotest (microplate), Mutatox test, the standard *Salmonella* plate-incorporation (Ames test), and the *Salmonella* fluctuation test, respectively (Legault *et al*, 1994).

Discriminativity:

Sediment particulates at high concentrations may suppress genotoxic reaction due to cytotoxicity. Such a background 'noise' interference became apparent when a relatively clean (being thoroughly washed in water) reference sediment was spiked with 4NQO and tested by the SOS Chromotest pad procedure. The resulting colour index values were considerably weaker at high sediment concentrations suspension (≥ 2.5%, w/v; Table 2). The suppression of colour development by sediment material was assumed to represent a cytotoxic effect, that interfered with the genotoxic reaction (i.e., the *de novo* synthesis of β -galactosidase enzyme and/or the enzymatic expression that develops the colour). Hence, in this bioassay, genotoxicity/toxicity must always be based on a reference point, i.e., by *comparison* between a test material to a

reference material. Accordingly, genotoxicity was determined in this study based on an *increase* in colour index induced by the test sediment sample as compared to a reference sediment. For example, genotoxicity was detected in the environmental sample Elbe #27 at sample concentration ranging from 5% to 0.31 (w/v), under the bioassay conditions (Table 4).

Cytotoxicity effect was based on a *decrease* in the colour index of the sample spiked with 4NQO, comparised to the 4NQO-spiked reference sample. The null assumption was that a greater cytotoxicity would cause a greater interference in the genotoxic reaction, thus resulting in a lower colour-index value. For example, substantial decrease in net-CIP of 4NQO-spiked samples, indicative of cytotoxicity, was detected in the Elbe #48 sample, at concentrations ranging from 10% - 0.16% (Table 4).

Consistency:

A good consistency of performance of the SOS-Chromotest pad procedure was demonstrated in repeat experiments in evaluating spiked and non-spiked reference sediment over five repeat experiments (genotoxicity values ranged 34 - 48, av. 40.6, coefficient of variance = 16%; Table 2) and environmental sediment samples over four repeat experiments (cytotoxicity values ranged from 24 - 33, av. 27.2, coefficient of variance = 15%; Table 3).

Simplicity:

The SOS-Chromotest pad is particularly easy to handle. The bioassay test organisms are bacteria that can be stored lyophilized for a long period of time (over 12 months at 4°C) without subculturing. The procedure protocol requires no special skills and minimal training. The bioassay colour endpoint is interpreted visually without a need for specialized equipment. Unlike other popular bacterial genotoxicity and mutagenicity bioassays which can only test liquids, or liquid-extract of solid samples (e.g., Ames test, *umu*C test or the Mutatox) the SOS-Chromotest pad procedure enables testing of solid-phase samples directly without extraction.

Cost:

The current cost of lyophilized bacteria, chromogenic pads and other consumables

is estimated to be $50 to $200 Canadian funds per sample, subject to the number of samples being tested at one time. The only accessory equipment items necessary are a 37°C incubator, and a simple spectrophotometer.

In conclusion, this study describes a new SOS-Chromotest pad procedure for testing of sediment samples for genotoxicity and cytotoxicity, directly without extraction. Preliminary data show that the bioassay is highly sensitive to direct-acting mutagens and can discriminates between genotoxic and cytotoxic pollutants. The bioassay procedure is easy to perform, requires minimal accessory equipment and cost effective. Further evaluations are required to determine the spectrum of detection of known direct-acting and indirect-acting mutagens, sensitivity levels, sample matrix limitations and consistency of performance of the new bioassay.

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TABLE 1: GENOTOXICITY EVALUATION OF 4-NITROQUINOLINE-N-OXIDE (4NQO) AT DIFFERENT CONCENTRATIONS IN WATER SOLUTIONS BY THE DIRECT SOS-CHROMOTEST PAD PROCEDURES, AT DIFFERENT INCUBATION TIMES

	Colour Index[1]											
	Incubation Time											
	Short[2] (7-hr) Repeat Experiments						Long[3] (24-hr) Repeat Experiments					
4NQO Concentration (ng/mL)	I	II	III	IV	V	VI	I	II	III	IV	V	VI
2,500.0	0	0	0	0	0	0	1	1	0	3	1	0
1,250.0	1	1	2	2	1	1	2	3	3	4	3	2
625.0	4	5	5	5	4	4	4	5	5	5	5	5
312.5	5	5	5	5	5	5	5	5	5	5	5	5
156.3	3	4	4	5	5	4	5	5	5	5	5	5
78.1	3	3	3	4	4	3	5	5	5	5	4	5
39.0	2	2	2	2	1	2	5	4	5	5	3	4
19.5	1	1	1	1	1	1	4	3	4	4	3	3
9.8	1	0	1	1	0	1	3	2	3	3	2	2
4.9	0	0	0	0	0	0	3	2	2	2	1	1
2.4	0	0	0	0	0	0	2	1	2	1	1	1
0 (Control)	0	0	0	0	0	0	1	1	1	1	1	1

1 Colour Index = ranged from 0 (no colour, no genotoxic effect detected) to 5 (very strong blue colour indicative of strong genotoxic effect).

2 The SOS-Chromotest Pad short procedure involved an exposure of sample solution to the test bacteria in a test tube for 4 hr at 37°C, followed by an additional 3-hr incubation period on a chromogenic pad at 37°C.

3 The SOS-Chromotest Pad long procedure involved an exposure of sample solution to the test bacteria in a test tube for 4 hr at 37°C, followed by an additional 20-hr incubation period on a chromogenic pad at 37°C.

TABLE 2: GENOTOXICITY EVALUATION OF A REFERENCE FRESH-WATER SEDIMENT BY THE DIRECT SOS-CHROMOTEST PAD PROCEDURE IN REPEAT EXPERIMENT

Repeat Experiment	CIP[1] non-spiked	CIP 4NQO-spiked[2]	net-CIP[3]	Genotoxicity Value[4]
I	(1)[5]00000012112	(1)12344455555	1**2344443443**[6]	35
II	(1)00000000011	(1)24455555555	**24455555544**	48
III	(1)00000011111	(1)22344555555	**22344544444**	40
IV	(1)00000000111	(1)13555555555	1**3555555444**	43
V	(1)00011100111	(1)02234455555	0**2223355444**	34

1 CIP = Colour Index Profile from 0 (no colour) to 5 (very strong colour) (one number per dilution).
2 4NQO-spiked = sediment sample was spiked with 4-Nitroquinoline-N-oxide (4NQO) at final concentration of 0.5 μg/g sediment (fresh weight).
3 net CIP = (CIP of 4NQO-spiked sediment) - (CIP of non-spiked sediment).
4 Genotoxicity Value = the sum total of the Net non-spiked CIP values which are ≥ 2.
5 Numbers in brackets represent control with no sediment.
6 Numbers in bold and underlined are the net-CIP values which are ≥ 2.

TABLE 3: GENOTOXICITY AND CYTOTOXICITY EVALUATION OF TWO SEDIMENT SAMPLES BY THE DIRECT SOS-CHROMOTEST PAD PROCEDURE IN REPEAT EXPERIMENTS

Sample ID	CIP[1] non-spiked	net-CIP non-spiked[3]	CIP 4NQO-spiked[2]	net-CIP 4NQO-spiked[4]	Genotoxicity Value[5]	Cytotoxicity Value[6]
Set I Reference	(1)[7] 00000012112		(1) 12344455555			
BD	(1) 00011122222	00011110110	(1) 00113455555	1222[8]1000000	0	6
BE	(1) 00000000111	00000000000	(1) 00000001344	12344454211	0	28
Set II Reference	(1) 00000000011		(1) 24455555555			
BD	(1) 00000011111	00000011100	(1) 01445555555	23010000000	0	5
BE	(1) 00000000111	00000000100	(1) 00000001455	24455553100	0	33
Set III Reference	(1) 00000011111		(1) 22344555555			
BD	(1) 00011111111	00011100000	(1) 14555555555	10000000000	0	0
BE	(1) 00000011111	00000000000	(1) 00000014555	22344541000	0	24
Set IV Reference	(1) 00000000111	00000000000	(1) 13555555555			
BD	(1) 00000000000	00000000000	(1) 01334555555	12221000000	0	6
BE	(1) 00000000000		(1) 00000001455	01334554100	0	24

1 CIP = Colour Index Profile from 0 (no colour) to 5 (very strong colour), one number per dilution.
2 4NQO-spiked = 4-Nitroquinoline-N-oxide (4NQO) was added at final concentration of 0.5 μg/mL suspension.
3 net non-spiked CIP = (CIP of non-spiked sediment) - (CIP of non-spiked reference sediment).
4 net spiked CIP = (CIP of 4NQO-spiked reference sediment) - (CIP of 4NQO-spiked sediment).
5 Genotoxicity Value = the sum total of the net non-spiked CIP values which are ≥ 2.
6 Cytotoxicity Value = the sum total of the net spiked CIP values which are ≥ 2.
7 Numbers in brackets represent control with no sediment.
8 Numbers in bold and underlined are net-CIP values which are ≥ 2.

TABLE 4: GENOTOXICITY AND CYTOTOXICITY EVALUATION OF FRESH-WATER SEDIMENTS BY THE DIRECT SOS-CHROMOTEST PAD PROCEDURE

Sample ID	CIP[1] non-spiked	net-CIP non-spiked[3]	CIP 4NQO-spiked[2]	net-CIP 4NQO-spiked[4]	Genotoxicity Value[5]	Cytotoxicity Value[6]
Set I Reference	(1)[7] 00000012112		12344455555			
Elbe #13	(1) 00000011111	00000000000	(1) 00112345555	1**2232**[8]110000	0	9
Elbe #18	(1) 00000101111	00000100000	(1) 01333345555	11011110000	0	0
Elbe #24	(1) 00011122222	00011110110	(1) 01234445555	11110010000	0	0
Elbe #27	(1) 14554322211	1**4554**310100	(1) 00134555555	1**22**10100000	21	4
Chile #8	(1) 00001111222	00001100110	(2) 04545555555	10000110000	0	0
Chile #12	(1) 00000201112	00000**2**00000	(1) 00234544444	1**2**110011111	2	2
Set II Reference	(1) 00000000011		(1) 24455555555			
Elbe #32	(1) 00000000000	00000000000	(1) 11244555555	1**32**11000000	0	7
Elbe #45	(1) 00000100000	00000100000	(0) 35555555555	**2**1100000000	0	2
Elbe #48	(1) 00000000000	00000000000	(1) 00000235555	**24455320**000	0	25
Elbe #53	(1) 00000100011	00000100000	(1) 45555555555	00000000000	0	0
Chile #9	(1) 00000100011	00000100000	(1) 34555555555	00000000000	0	0

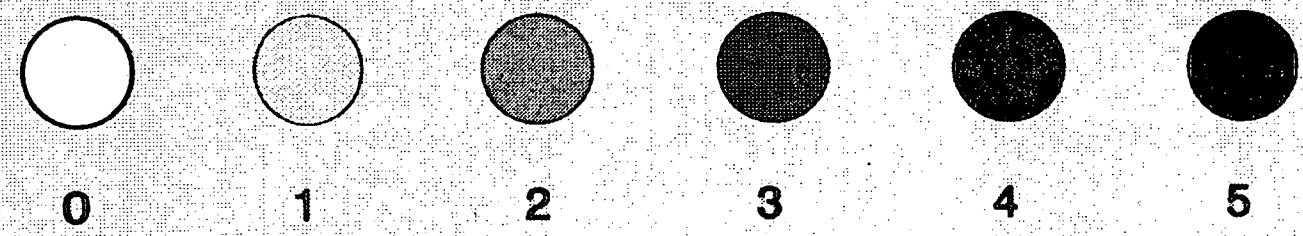
1 CIP = Colour Index Profile from 0 (no colour) to 5 (very strong colour), one number per dilution.
2 4NQO-spiked = 4-Nitroquinoline-N-oxide (4NQO) was added at final concentration of 0.5 μg/mL suspension.
3 net non-spiked CIP = (CIP of non-spiked sediment) - (CIP of non-spiked reference sediment).
4 net spiked CIP = (CIP of 4NQO-spiked reference sediment) - (CIP of 4NQO-spiked sediment).
5 Genotoxicity Value = the sum total of the net non-spiked CIP values which are ≥ 2.
6 Cytotoxicity Value = the sum total of the net spiked CIP values which are ≥ 2.
7 Numbers in brackets represent control with no sediment.
8 Numbers in bold and underlined are net CIP values which are ≥ 2.

FIGURE LEGEND

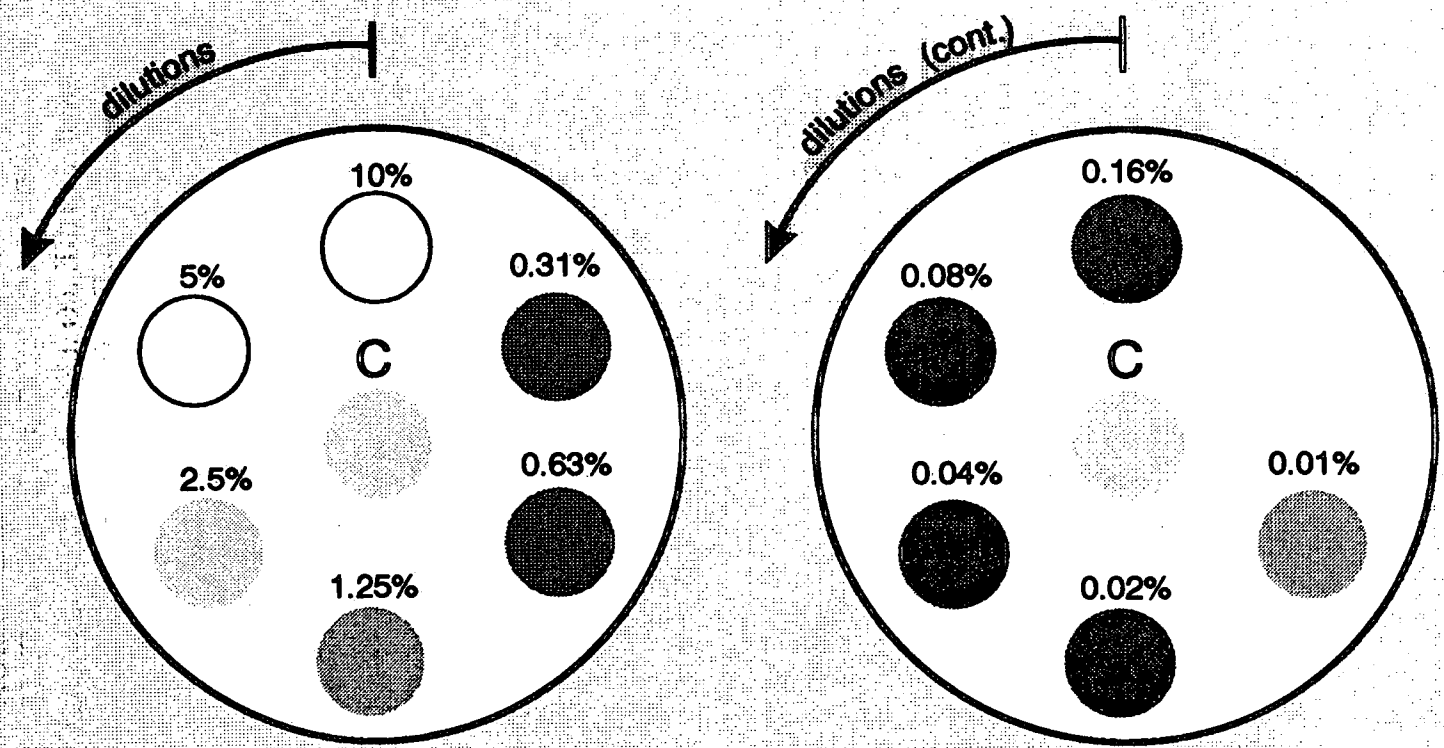
Figure 1:

An illustration of the bioassay colour-index and an example of sample layout on pads. The spot at the centre of each pad (marked C) is the control (no sediment added). The colour index-profile (CIP) of this example is recorded as (1) 00123344442.

Colour Index



Sample Layout on Pads



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