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APPLICATION AND COMPARISON OF THE ECHA BIOCIDE MONITOR IN ENVIRONMENTAL SAMPLES

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ABSTRACT

A procedure originally designed to test the effectiveness of <u>in situ</u> biocides was evaluated as a possible environmental toxicity assessment procedure. The ECHA Biocide Monitor was applied to 94 sediment extracts and compared to three well documented toxicant assessment procedures; Microtox, <u>Daphnia magna</u> and <u>Spirillum volutans</u>. Results of this investigation are detailed.

RÉSUMÉ

Pour évaluer des substances toxiques dans des échantillons environnementaux, nous avons étudié la possibilité d'utiliser une technique qui devait, à l'origine, servir à vérifier l'efficacité de biocides *in situ*. La technique de contrôle des biocides ECHA (ECHA Biocide Monitor) a été appliquée à 94 échantillons de sédiments et comparée à trois techniques d'évaluation de substances toxiques bien documentées : Microtox, *Daphnia magna* et *Spirillum volutans*. Les résultats de cette étude sont présentés en détail.

EXECUTIVE SUMMARY

In searching for the components of an ideal "battery of tests" to screen for toxicant activity in environmental samples, we are always open to investigating similar type procedures used in different fields. The ECHA Biocide Monitor kit is a commercial preparation for evaluating disinfectant efficiency and is inexpensive and simple to perform which might be adaptable to environmental samples.

Studies on the feasibility of the ECHA Biocide Monitor to screen environmental samples indicate that it has potential for on-site evaluation and can be performed by anyone with no special training. We believe with a larger data base this procedure could become part of all field survey/monitor studies to support selection of appropriate samples and sampling sites.

RÉSUMÉ

Dans notre recherche d'éléments d'une batterie de tests idéale servant à détecter une activité toxique dans des échantillons environnementaux, nous sommes toujours disposés à évaluer les méthodes similaires utilisées dans d'autres domaines. La trousse de contrôle des biocides ECHA (ECHA Biocide Monitor Kit) est un produit commercial utilisé pour évaluer l'efficacité d'un désinfectant. Il s'agit d'un produit peu coûteux et facile à utiliser qui pourrait s'adapter aux échantillons environnementaux.

Les études sur l'utilisation de la trousse de contrôle des biocides ECHA pour vérifier les échantillons environnementaux montrent que cette méthode peut servir pour effectuer des évaluations sur place et qu'aucune formation spéciale n'est nécessaire pour l'appliquer. Nous croyons qu'avec une base de données plus grande, cette méthode pourrait faire partie de toutes les études de contrôle sur le terrain et faciliter le choix d'échantillons et de points d'échantillonnage appropriés.

INTRODUCTION

A variety of test methods, criteria and procedures have been developed internationally to assess the impact of chemical pollutants. With the increasing awareness of the long-term effects of chemicals discharged into aquatic systems, research efforts are being directed at short-term bioassay tests to alert monitoring agencies as well as dischargers of toxic conditions (Bulich and Green, 1979; Dutka and Kwan, 1980). Another goal of short-term bioassay development is to prioritize samples, both sediment and water, for detailed chemical analyses. The majority of short-term bioassay tests which are simple and do not require sophisticated technology are microbial-based and often are specific to individual laboratories or enterpreneurs.

In our review of this field, we became aware of the ECHA Biocide Monitor[1,2]. This monitor was originally developed as a "dip-stick" method for on-site evaluation of biocide concentrations and effectiveness. The simplicity and inexpensiveness of the ECHA Biocide Monitor and its potential for application to environmental monitoring were quickly noted and the decision was made to evaluate it with environmental samples. In this evaluation, the sensitivity of the procedure was compared to results obtained from the following well documented toxicant screening tests; 48 hr Daphnia magna (APHA, 1985), Microtox (Bulich and Greene, 1979) and Spirillum volutans (Dutka and Kwan, 1982). The results of this preliminary study are presented.

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(1) E.C. Hill and Associates, Cardiff, U.K.

(2) Use of trade name or product does not imply endorsement of the product or service by Environment Canada.

METHODS

Samples

A total of 94 sediment samples collected from widely separated points in Canada were used to evaluate the feasibility of using the ECHA Biocide Monitor to screen for toxicant activity in environmental samples. Sediments were collected from Port Hope harbour and surrounding areas in Lake Ontario, Fraser River (British Columbia Province), Saskatchewan River (Alberta and Saskatchewan Provinces), Tobin Lake (Saskatchewan Province) and lakes in the Province of Manitoba. These sediments were water-extracted at 1 mL Milli Q water to 1 gram wet weight sediment (Dutka et al., 1987) and the extract was used for toxicant activity measurement.

TOXICANT SCREENING TESTS

The Microtox test was performed using the luminescent acterium Photobacterium phosphoreum and the procedure detailed in Beckman Microtox Operation Manual (1982) with a 15-minute contact time (Dutka and Kwan, 1981).

Spirillum volutans, a large bacterium with a rotating fascicle of flagella at each end, was used to test the extracts for toxicity, following a modification of the procedure developed in 1974 by Boudre and Krieg (Dutka, 1986).

Ten Daphnia magna per 25 mL of sample (and sample dilution) were used to test each sediment extract following procedures detailed in APHA Standard Methods (1985).

The ECHA Biocide Monitor is based on the use of a small absorbent pad impregnated with a sensitive test organisms (Bacillus species) and a growth indicator dye (tetrazolium) to detect the presence of microbial growth. For the test, the absorbent pad is dipped into the sample or dilution of sample to be tested for approximately 10 seconds. Surplus fluid is drained off, and the strip is transferred to its individual incubation chamber. The labelled chamber is incubated at 35-37°C for 18-24 hours. Results can be observed on the pad and are interpreted according to the colour card enclosed with the test kit. A toxic sample produces a white pad, and a nontoxic sample produces a red pad. Pink or spotted pads indicate ± or doubtful ranges. By following colour changes, close range end points can be obtained.

RESULTS AND DISCUSSION

Data on the sensitivity of the ECHA Biocide Monitor to various biocides is readily available from the manufacturer and is distributed as an ECHA Biocide Monitor Data Sheet #20.7.87 F53. For instance, the following sensitivities are reported: Aciticide AZ, 30 ppm; Bioban CS-1248, 120 ppm; Biomate 5797, 10-100 ppm; Bodoxin 25 ppm; Formaldehyde 40% w/v, 75 ppm; Grotan TK2, 50 ppm; and Myacide AS, 11 ppm. The manufacturer also indicated that the system does have blind spots. It is not very sensitive to some chlorinated phenolics and is inhibited only by concentrations much greater than those in practical use. Another blind spot occurs with biocides which are active because they are oxidizing agents (chlorine and chlorine release components). The activity of these biocides tends to get "mopped up" by the absorbent pad and its nutrients (manufacturer's brochure).

Before embarking on a comparison programme, we required some information on the sensitivity of the ECHA Biocide Monitor to a chemical which has been tested by a variety of toxicant screening tests. To this end, Hg^{++} was selected and tested in the form of $HgCl_2$. Data produced by Dutka and Kwan (1982) indicated that the 15 min Microtox test had an EC_{50} of 0.046 ppm Hg and the 120 min Spirillum volutans produced a toxic effect at 0.2 ppm Hg. With the ECHA Biocide Monitor a toxicant effect was found when the Hg^{++} concentration was between .5 and .6 ppm, not an unreasonable expectation of sensitivity.

Table 1 presents a summary of those sediment extracts which produced positive (toxicant) or partially positive (± white with red spots) responses. It can be seen that 27 of the 94 samples produced a measureable effect with the ECHA Biocide Monitor, and 16 of these were also positive by the Daphnia magna test. Surprisingly, only one sample was positive with the Spirillum volutans test and no samples produced an EC_{50} effect in the Microtox test. Clearly, it is likely that each toxicant assessing procedure is responsive to different mixes and concentrations of chemicals in these sediment extracts. The Daphnia magna test appears to have sensitivity patterns somewhat similar to, but much more sensitive than the ECHA Biocide Monitor.

Every test which produced a positive or ± effect in the ECHA strip also produced an effect in the Daphnia magna test. There were 11 occasions (Table 1) where the ECHA Biocide Monitor indicated toxicant activity while the Daphnia magna test indicated no toxicant was present. However, when all 94 samples were compared a total of 63 samples produced a toxic effect in the Daphnia magna test compared to only 27 with the ECHA Biocide Monitor.

The data shown in Table 1 are very supportive of the belief expressed by many researchers that it is dangerous and unwise to try and assess the presence of toxicants in waters, effluents or sediments by the use of a single toxicant screening procedure. The "battery of tests" approach using three or four tests with different types of end points (i.e. loss of fluorescence, loss of motility, inhibition of growth, inhibition of ATP production, inhibition of O_2 uptake or death) should be used to assess toxicant presence and activity.

Data obtained from the 94 sediment extracts indicate that the ECHA Biocide Monitor has the potential for on-site testing and priority screening of samples for toxicant activity. To this end, some minor studies were carried out to investigate the effect of increased contact time between the dipstick and sample.

Six sediments collected from Hamilton Harbour were used in this study. Distilled Milli Q water was used as a control. Three dipsticks were placed 1 cm below the surface of each sample. One dipstick was removed from each sample after one, 10 and 60 minutes of exposure. Excess sediment was removed from each dipstick, but no rinsing was done. After overnight incubation (16-18 hr) each dipstick was rinsed off in distilled water and the colour of each bacterial pad compared to that provided with the ECHA Kit. The results are shown in Table 2.

Table 2. Summary of results of ECHA Biocide dipstick test in relation to contact time with sediment samples

Contact Time	Sample No.						
	1	2	3	4	5	6	Milli Q H_2O
1 minute	+	±	-	+	±	-	-
10 minutes	-	±	-	±	±	±	-
60 minutes	+	+	±	+	+	+	±

There appears to be an intensifying of response with increased contact time in four of the six samples which suggests that environmental samples, such as sediments, with low levels of toxicants may be detected with increased exposure time.

The same six sediments were extracted 1:1 with Milli Q water similarly to those in Table 1. Interestingly all six sediment extracts failed to produce a positive (toxicant presence) test with the ECHA Biocide Monitor dipstick. Thus the direct application of the dipstick into sediment and withdrawing and incubating the dipstick with its adhering sediment may produce positive (toxic) effects due to the presence of toxicants within the sediment structure. The study on increased contact time suggests that the dipstick procedure has environmental toxicant assessment potential. There is also a suggestion that the sensitivity of the dipstick method in evaluating sediments may be increased by incubating (at 35°C) a portion of the sediment with an immersed dipstick for periods varying from one hour to overnight. More research into this potential application of the ECHA Biocide Monitor dipstick is required.

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Table 1. Summary of sediment extract samples that indicated the presence of toxicants by the ECHA Biocide Monitor dipstick.

Sample Number	ECHA Dipstick	*Daphnia magna* 48 hr test	Microtox 15 min test	*Spirillum volutans* 120 min test
A	±	EC_{40} at 100%	neg	neg
B	+	EC_{20} at 100%	neg	neg
C	±	EC_{50} at 50%	neg	neg
L	±	neg	neg	neg
N	+	neg	neg	neg
O	+	neg	neg	neg
1	±	EC_{40} at 75%	neg	neg
3	±	neg	neg	neg
6	+	EC_{30} at 100%	neg	neg
7	±	neg	neg	neg
15	±	neg	neg	neg
16	±	neg	neg	neg
17	±	neg	neg	neg
19	±	neg	neg	neg
25	+	EC_{50} at 31%	neg	neg
28	+	EC_{50} at 31%	neg	neg
34	±	EC_{40} at 100%	neg	neg
38	+	EC_{20} at 100%	neg	neg
48	+	EC_{20} at 100%	neg	neg
49	+	EC_{20} at 100%	neg	neg
50	±	neg	neg	neg
51	±	EC_{30} at 100%	neg	neg
55	±	EC_{50} at 78%	neg	neg
63	+	not done	neg	+
65	±	EC_{50} at 92%	neg	neg
69	±	EC_{50} at 82%	neg	neg
72	+	EC_{50} at 92%	neg	neg
#Positive	27	16	0	1
Total Positive	27/94	63/93	0/94	1/94