Rapid detection of toxicity in water using the oxygen uptake rate of mammalian cells as sensor*

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Abstract

A short-term bioassay test procedure for the detection of toxicity in water was adapted and evaluated. The test is based on the effect of toxicants on the oxygen uptake rate of cells of the Buffalo green monkey (BGM) kidney cell line. The test yields results within 10 min and was more sensitive to the majority of selected toxicants than the equally rapid *Tetrahymena pyriformis* oxygen uptake test. Although not sufficiently sensitive for general screening of drinking-water supplies, it has attractve features for certain purposes such as the rapid testing of wastewaters and industrial effluents.

Introduction

Biological methods have been used for many years to detect toxic substances in water (Little, 1976; Hunter, 1978). While identification of toxicants depends largely on chemical analyses (Trussel and Umphres, 1978; Hattingh, 1979), bioassays are excellent tools for general screening of water for toxic effects. Various members of the aquatic food chain, including fish, metazoa, algae, protozoa and bacteria, have been used in water toxicity studies (Little, 1976; Kingsbury and Rees, 1978), using methods based on the survival of organisms and physiological functions such as growth, reproduction, metabolism and behaviour (Little, 1976; Hunter, 1978).

Recently, considerable attention has been given to the development of short-term bioassays which will rapidly detect toxicants with the same sensitivity as long-term tests (Bulich and Greene, 1979; Liu, 1981; Williamson and Johnson, 1981). Bacteria and protozoa have been found particularly suitable for these methods because of their short generation times and easily observed, rapid response to environmental changes. Toxicity has been measured using parameters such as bioluminescence (Bulich and Green, 1979), nitrification (Williamson and Johnson, 1981) and enzyme activity (Liu, 1981). We developed a bioassay technique based upon oxygen uptake rate of the protozoan *Tetrahymena pyriformis* (Slabbert and Morgan, 1982). This technique was successful in detecting chronic levels of individual toxicants within 10 min. Toxic effects were measured using a biological oxygen monitoring system. Unfortunately, like most procedures utilizing aquatic organisms as indicators of toxicity, the technique did not prove sufficiently sensitive to monitor the quality of drinking-water.

This shortcoming has been overcome by the development of mammalian cell culture techniques, which provide a sensitive system for the detection of toxic substances in water. A technique based upon the cloning efficiency of mammalian cells (Kfir and Prozesky, 1981) proved to be twice as sensitive to known toxicants as an electronic fish biomonitoring system (Morgan, 1977). Using buffalo green monkey (BGM) cells, derived from non-tumorous kidney tissue, the technique was found sufficiently sensitive to evaluate the quality of drinking-water (Kfir and Prozesky, 1982a). In the present study BGM cells were used as biological material in the rapid bioassay technique based upon oxygen uptake rate (Slabbert and Morgan, 1982) in an attempt to improve its sensitivity. A number of known toxicants were tested in order to evaluate the sensitivity of the system. The ability of the system to detect toxicity in water samples has also been investigated.

Materials and Methods

BGM kidney cell line

Cells originally obtained from Mr D.R. Dahling, U.S. Environmental Protection Agency, Cincinnati, Ohio, were cultured in 275 mℓ tissue culture flasks as described earlier (Grabow and Nupen, 1981). Growth was confluent within three to four days.

Preparation of cells for bioassaying

Cells were prepared at room temperature under non-sterile conditions. Cells (confluent cultures) were trypsinized using 5 mℓ of a 1,25 g/ℓ trypsin (Difco 1:250) solution in phosphate buffer (PBS) for 10 to 15 min. The cell contents of five flasks (sufficient for one toxicity test) were collected in a conical centrifuge tube containing 2 mℓ of foetal bovine serum to inactivate the trypsin. Flasks were rinsed with 10 mℓ of a 8,5 g/ℓ saline solution. Cells were centrifuged at 2 000 r/min for 1 min, washed in 40 mℓ of saline solution (per tube) and resuspended in 5 mℓ of the same solution to yield an oxygen uptake rate of approximately 5 %/min (Figure 1, line a). The cells were transferred to a clean container and kept in suspension using a magnetic stirrer. The saline solution provided a suitable osmotic balance for the cells without having a chemical effect on the toxicants. Cells were allowed 15 min to stabilize before use within 1 h of preparation.

Oxygen monitoring apparatus

A biological oxygen monitoring system (YSI model 53, Yellow Springs Instrument Co., Yellow Springs, Ohio) consisting of a standard bath unit equipped with airtight test chambers and a built-in magnetic stirrer, an oxygen probe fitted in a plunger provided with a slanting front and an access slot along one side,

*Revised paper, originally presented at Symposium on Water Research Selected In-depth Studies jointly organized by the National Institute for Water Research and the Southern African Branch of the Institute of Water Pollution Control, 18 November 1982.

closely sliding into test chambers, and an electronic unit, was employed to examine the effect of toxicants on the oxygen uptake rate of BGM cells. The temperature of the system was stabilized at 27 °C using a constant temperature water circulator (Lauda K2R electronic, Messgeräte-Werk Lauda/Tauber, FDR). A potentiometric recorder (Linear model 355, Linear Instrument Corp., Irvine, California) was linked to the monitoring system to provide a permanent graphic record of oxygen uptake rate, the chart speed being regulated to 1 200 mm/h. The apparatus was calibrated using air-saturated deionized water.

Test samples

Individual toxicants – Test solutions of copper ($CuSO_4$), cadmium ($CdCl_2$), mercury ($HgCl_2$), lead ($PbCl_2$), cyanide (NaCN), ammonia-N ($(NH_4)_2SO_4$) and phenol (C_6H_5OH) were prepared with deionized water. Toxicants were prepared as twentyfold concentrates in order to obtain the required test concentration (Table 1) after addition to cell suspensions.

Water samples – Single samples of laboratory tap water, settled sewage, humus tank and activated sludge effluent (Daspoort sewage works, Pretoria) and an industrial effluent (Germiston) were taken. Sewage samples and the industrial effluent were decontaminated by means of filtration through a 0,45 μm Sartorius membrane filter (Slabbert, Smith and Morgan, 1983).

Bioassaying

Tests were carried out according to the procedure described by Slabbert and Morgan (1982). For each test, 5 mℓ of the BGM cell suspension was aerated for 5 min in a test tube maintained at 27 °C; 4,75 mℓ of the suspension was then transferred to a test chamber provided with a magnetic stirrer, the oxygen probe inserted and all air bubbles expelled. Aeration was carried out outside the test chamber because of the formation of foam. Test solutions (0,25 mℓ) were introduced when dissolved oxygen in the chamber had decreased to between 60 and 65%. Samples were injected through the access slot in the plunger using a microsyringe with a long needle. Oxygen uptake was recorded continuously before (reference), during (mixing) and after (test) sample addition (Figure 1).

The effect of toxicants on oxygen uptake rate was established by comparing test uptake rates with reference uptake rates, expressing all results in relation to a standard reference uptake rate of 5%/min. Results were determined at 1, 2, 3, 4 and 5 min after mixing by drawing tangents to the graphs at these times. Four tests were carried out with each toxicant concentration (or water sample). Deionized water was used for control tests. Student's t-test was applied to establish whether average test results differed significantly from average control results at the $P = 0,05$ level.

Results and Discussion

BGM cells showed a constant oxygen uptake rate until dissolved oxygen had decreased to approximately 50%, thereafter a gradual decrease occurred (Figure 1, line a). A reference oxygen uptake rate of 5%/min allowed sufficient time before sample addition (3 to 4 min) to examine oxygen uptake. Mixing of test samples and cell suspensions was recorded as a line parallel to the time axis (Figure 1, line b). The addition of deionized water to cell suspensions caused a change in uptake rate because of a dilution effect on cell concentration (Figure 1, line c). This graph was used as a convenient control for test graphs. Toxicants caused an increase (Figure 1, line d) or a decrease (Figure 1, lines e and f) in the oxygen uptake rate of cells. Test uptake rates in excess of or less than control rates were thus attributed to toxic activity in the test sample.

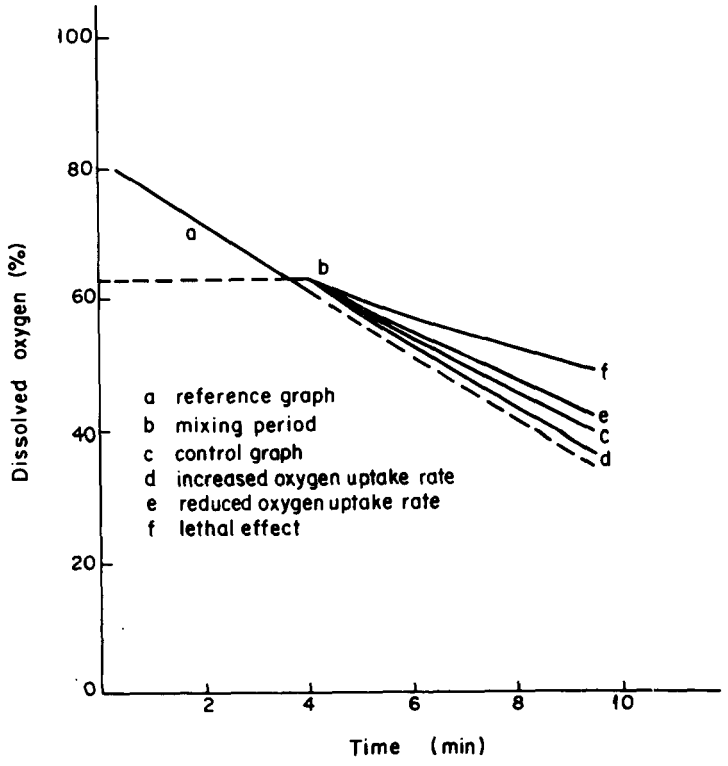

Figure 1
Typical effects of toxicants on the oxygen uptake rate of BGM cells

TABLE 1
THE EFFECT OF INDIVIDUAL TOXICANTS ON THE OXYGEN UPTAKE RATE OF BGM CELLS

Toxicant	Concentration (mg/ℓ)	Oxygen uptake rate at time indicated (min) 1	2	3	4	5
Cu^{2+}	5,0*	4,06	4,06	4,06	4,05	4,02
	1,0*	4,31	4,31	4,29	4,29	4,29
	0,5	4,65	4,65	4,63	4,60	4,56
	0,1	4,49	4,49	4,47	4,43	4,40
	0,05	4,36	4,36	4,36	4,36	4,34
	Control	4,41	4,41	4,40	4,37	4,36
Cd^{2+}	5,0*	4,19	4,19	4,19	4,16	4,14
	1,0*	4,23	4,23	4,20	4,18	4,18
	0,5	4,57	4,56	4,53	4,50	4,47
	0,1	4,57	4,57	4,56	4,55	4,53
	0,065	4,41	4,41	4,41	4,39	4,38
	Control	4,42	4,42	4,40	4,38	4,37
Hg^{2+}	10,0*	4,06	4,06	3,93	3,86	3,86
	1,0	4,31	4,31	4,31	4,25	4,23
	0,5	4,51	4,50	4,47	4,45	4,41
	0,1	4,48	4,48	4,46	4,46	4,46
	Control	4,41	4,41	4,40	4,38	4,37
Pb^{2+}	10,0*	4,05	4,05	4,05	4,02	4,02
	1,0	4,22	4,22	4,19	4,16	4,13
	0,5	4,41	4,41	4,40	4,36	4,33
	0,1	4,43	4,43	4,43	4,42	4,39
	Control	4,37	4,37	4,37	4,35	4,34
CN^-	0,5*	3,32	3,05	2,83	2,72	2,66
	0,05	4,23	4,23	4,22	4,21	4,20
	0,01	4,35	4,35	4,35	4,35	4,33
	Control	4,35	4,35	4,35	4,34	4,33
NH_3-N	50,0*	5,00	5,00	4,88	4,75	4,70
	5,0	4,53	4,53	4,51	4,47	4,44
	0,5	4,40	4,40	4,38	4,38	4,37
	Control	4,36	4,36	4,35	4,33	4,33
C_6H_5OH	100	3,91	3,91	3,91	3,90	3,89
	50	4,01	4,01	4,00	3,97	3,96
	10	4,28	4,28	4,28	4,27	4,27
	1	4,42	4,42	4,42	4,42	4,42
	Control	4,39	4,39	4,39	4,39	4,38

Results are expressed in relation to a reference oxygen uptake rate of 5%/min. Each result is an average of four repetitions except those marked with an asterisk

Table 1 summarizes the effect of various toxicant concentrations upon the oxygen uptake rate of BGM cells. Concentrations used in the study were based on levels detected with the *T. pyriformis* oxygen uptake bioassay (Slabbert and Morgan, 1982) and the cloning efficiency technique (Kfir and Prozesky, 1981). Results are expressed in relation to a reference oxygen uptake rate of 5%/min and represent the mean values of four determinations. The coefficient of variation for test and control results generally varied from 0 to 2%. In cases of 0,5 mg/ℓ copper and cadmium, 1,0 mg/ℓ mercury and lead, and 100 mg/ℓ phenol, however, variation was slightly greater (coefficient of variation: up to 3%).

Controls showed an uptake rate of between 4,42 and 4,35 % at 1 min after mixing, to between 4,38 and 4,33 % at 5 min after mixing. Copper, cadmium and mercury affected oxygen uptake rate of BGM cells in a similar way, lower concentrations causing an increase in uptake rate, and higher concentrations a reduction. The effect of copper on oxygen uptake rate of the cells is illustrated in Figure 2. Ammonia-N increased oxygen uptake rate to a greater or lesser extent, depending on the concentration employed. Test concentrations of lead, cyanide and phenol (Figure 3) only caused a reduction in oxygen uptake rate, the higher the concentration the larger the reduction. The effect of copper, cadmium, cyanide and phenol on oxygen uptake rate of BGM cells resembled the effect of the toxicants on the oxygen uptake rate of *T. pyriformis* (Slabbert and Morgan, 1982). Mercury and ammonia-N only caused a reduction in the oxygen uptake rate of *T. pyriformis* while low concentrations of lead increased the uptake rate.

Lowest test concentrations of the toxicants affecting oxygen uptake rate of BGM cells are shown in Table 2. The cells were most sensitive to cyanide, followed by the metals, ammonia-N

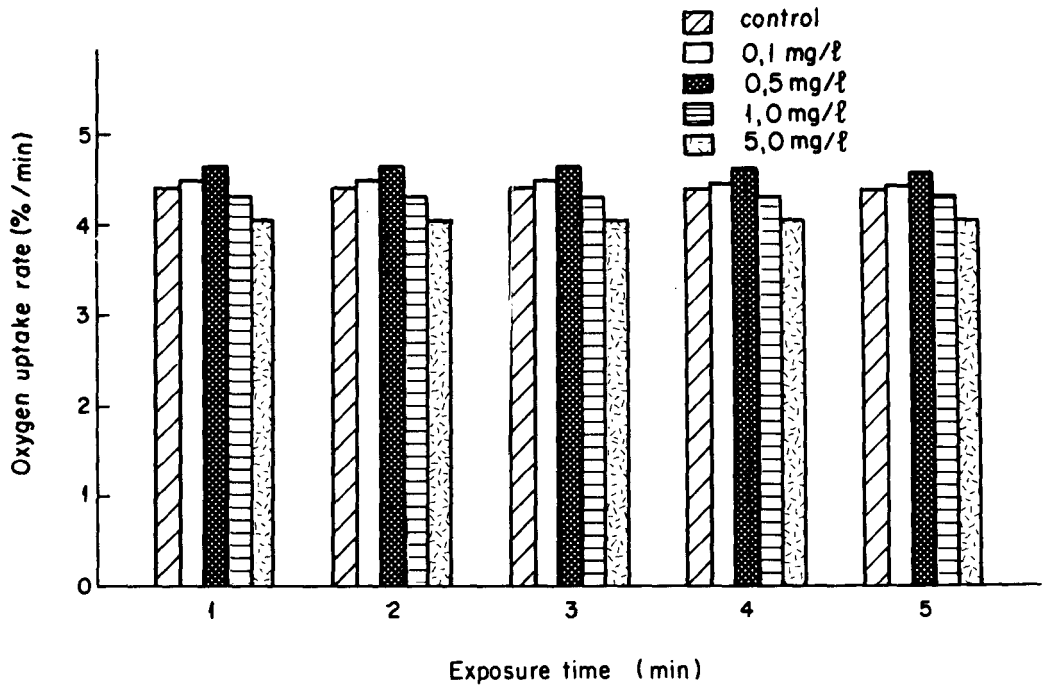

Figure 2
The effect of copper on the oxygen uptake rate of BGM cells

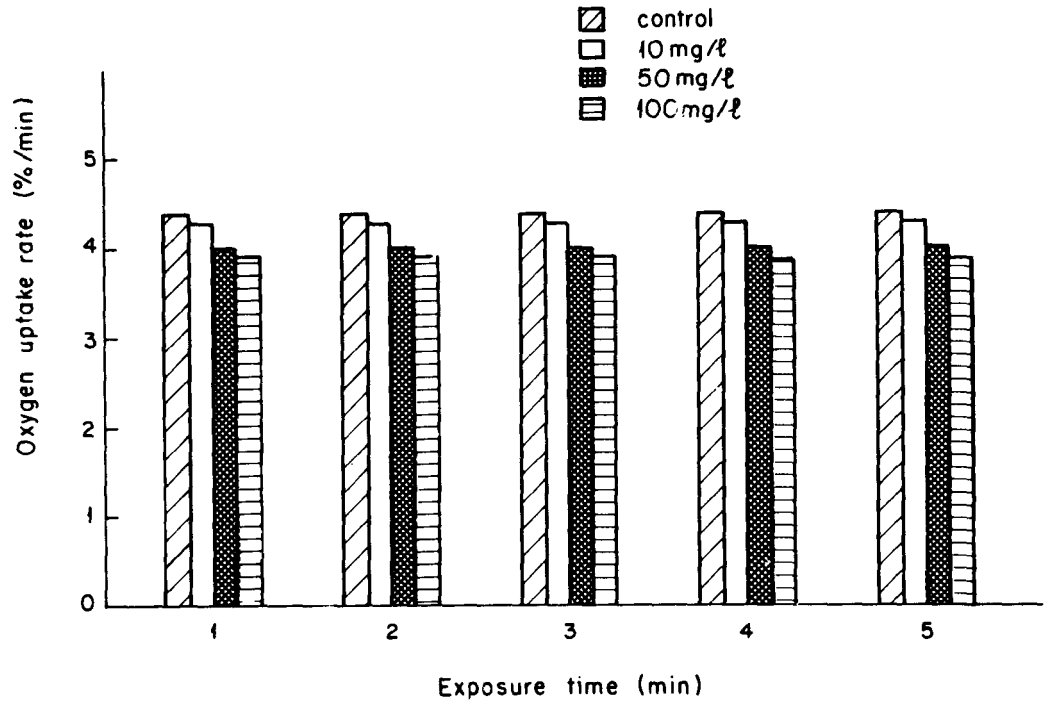

Figure 3
The effect of phenol on the oxygen uptake rate of BGM cells

TABLE 2
LOWEST TEST CONCENTRATIONS OF INDIVIDUAL TOXICANTS AFFECTING THE OXYGEN UPTAKE RATE OF BGM CELLS, IN COMPARISON WITH LOWEST CONCENTRATIONS DETECTED WITH THE *TETRAHYMENA PYRIFORMIS* OXYGEN UPTAKE BIOASSAY[1], CONCENTRATIONS DETECTED WITH THE CLONING EFFICIENCY TECHNIQUE[2], *INTERNATIONAL STANDARDS FOR DRINKING WATER*[3] AND THE *GENERAL STANDARD FOR TREATED INDUSTRIAL EFFLUENT*[4]
(concentration in mg/ℓ)

	Lowest test concentration affecting oxygen uptake rate of BGM cells	Lowest concentration affecting oxygen uptake rate of *T. pyriformis*	Concentration affecting cloning efficiency of BGM cells[5]		WHO international standards for drinking water	General standard for treated industrial effluent
Cu^{2+}	0,1	0,5	0,025	(69,8)	0,05	1,0
Cd^{2+}	0,1	0,5	0,065	(30,4)	0,01	[6]
Hg^{2+}	0,5	0,5	0,005	(42,1)	0,001	[6]
Pb^{2+}	1,0	0,01	0,5	(62,3)	0,1	1,0
CN^-	0,05	0,014	[6]		0,05	0,5
NH_3-N	5,0	275	0,5	(78,8)	[6]	10,0
C_6H_5OH	10,0	90	0,5	(67,6)	0,001	0,1

[1]Slabbert and Morgan (1982)
[2]Kfir and Prozesky (1981)
[3]World Health Organization (1971)
[4]South Africa, Republic (1962)
[5]% survival indicated in brackets
[6]Not available

TABLE 3
EFFECT OF WATER SAMPLES ON OXYGEN UPTAKE RATE OF BGM CELLS

Water sample	Oxygen uptake rate at time indicated (min) 1	2	3	4	5
Settled sewage	4,54	4,54	4,52	4,48	4,46
Humus tank effluent	4,42	4,42	4,39	4,38	4,35
Activated sludge effluent	4,38	4,38	4,37	4,37	4,37
Control	4,40	4,40	4,39	4,39	4,38
Tap water	4,40	4,40	4,38	4,38	4,38
Industrial effluent	5,78	5,70	5,59	5,30	5,22
Control	4,37	4,37	4,37	4,36	4,36

Results expressed in relation to a reference oxygen uptake rate of 5%/min. Each result is an average of four repetitions

and phenol. A comparison with lowest concentrations detected with the *T. pyriformis* oxygen uptake bioassay (Slabbert and Morgan, 1982) indicated that BGM cells were much more sensitive to ammonia-N (~ 55 times), slightly more sensitive to phenol (~ 9 times), copper and cadmium (~ 5 times) and approximately 100 times less sensitive to lead. The sensitivity to mercury was the same, and to cyanide approximately the same. No toxicants could be detected at concentrations as low as those detected by the cloning efficiency technique (Kfir and Prozesky, 1981). Since lowest concentrations affecting cloning efficiency were not determined, a direct comparison between the sensitivities of the two methods was not possible. With both methods (oxygen uptake bioassay and the cloning efficiency test) BGM cells showed a higher sensitivity to metals than to phenol and ammonia-N. The cells were less sensitive to lead than to the other metals. The great difference in sensitivity between the techniques may be attributed to the difference in the exposure period of BGM cells, namely 5 d in the case of cloning efficiency against only 5 min during oxygen uptake tests. All toxicants except phenol could be detected using the oxygen uptake rate of BGM cells at concentrations lower than or equal to the general standard for treated industrial effluent (South Africa (Republic), 1962). Cyanide alone was detected at a concentration complying with the standard for drinking-water set by the World Health Organization (WHO, 1971).

Data on the effect of water samples on the oxygen uptake rate of BGM cells are given in Table 3. Only one sample of each type of water was tested. The coefficient of variation for all results except those for industrial effluent (coefficient of variation: up to 4%) was less than 2%. In order to compare the sensitivity of BGM cells with that of *T. pyriformis* the same samples were tested using the *T. pyriformis* oxygen uptake bioassay (Slabbert and Morgan, 1982). The mammalian cells and *T. pyriformis* responded in exactly the same way to the various types of water, humus tank effluent, activated sludge effluent and tap water having no effect, whereas settled sewage and industrial effluent caused an increase in oxygen uptake rate. The industrial effluent produced a much greater increase in the oxygen uptake rate of both BGM cells and *T. pyriformis* than settled sewage, indicating higher toxicity (Slabbert, Smith and Morgan, 1983). Toxic activity was detected in all the wastewaters tested by means of the cloning efficiency test (Kfir and Prozesky, 1981), settled sewage being extremely toxic. The lower sensitivity of the oxygen uptake rate tests to these samples may, among other factors, be attributed to the dilution effect inherent in the technique. Drinking-water showed no or minimal toxic activity when tested by the cloning efficiency technique (Kfir and Prozesky, 1982a).

Conclusions

The BGM cell oxygen uptake test was successfully employed as a rapid screening test for toxicants in water. The degree of toxic effect was clearly indicated and results showed good reproducibility (the coefficient of variation usually varied between 0 and 2%). The costs involved in conducting the tests could be a major drawback because large amounts of cells are required for each test, involving the use of many disposable tissue culture flasks and quantities of expensive media. This problem may be solved, to a large extent, by using mass culturing procedures involving the growth of cells in suspension. This would at the same time facilitate the easier preparation of cell suspensions for bioassaying.

Although lower concentrations of some of the individual toxicants could be detected, the sensitivity of the technique to the total toxicant concentration of a limited number of water samples did not appear to be any better than that of the *T. pyriformis* bioassay system. The technique did not prove to be sufficiently sensitive to monitor the quality of drinking-water in terms of WHO limits for the toxicants tested. However, it could be very useful for the rapid screening of drinking-water supplies accidentally or deliberately contaminated with relatively high concentrations of a wide variety of toxicants. In view of the response of the BGM cell oxygen uptake test to wastewaters and selected toxicants, and the limits for industrial effluents listed in Table 2, it may be applied successfully for the rapid screening of industrial effluents and wastewaters.

Compared with other short-term bioassays, the BGM cell oxygen uptake test has the advantage, provided cells are available, that it yields results within 10 min and is more sensitive to the majority of the selected toxicants than the equally rapid *T. pyriformis* bioassay. The mechanism of action involved in short-term toxicity assays such as the BGM cell and *T. pyriformis* oxygen uptake tests will, of course, not permit the detection of potentially chronic effects on man and other animals resulting from long-term exposure to low levels of certain toxicants. These tests are also not suitable for the detection of mutagenic and potentially carcinogenic compounds, for which other short-term assays such as the Ames *Salmonella* mutagenicity (Grabow, Denkhaus and Van Rossum, 1980) and the mammalian cell transformation (Kfir and Prozesky, 1982b) assays have been designed. It follows that the overall biological screening of the chemical quality of water requires a battery of test systems in which each has a particular objective.

Acknowledgements

Thanks are due to Dr W.S.G. Morgan, Mrs E.M. Nupen and Dr W.O.K. Grabow for advice and Mrs W.L. Strydom for technical assistance.

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