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Toxicity of Benzoquinone and Hydroquinone in Short-Term Bacterial Bioassays

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Use of bioassays to assess the toxicity of pollutants is an important component of environmental testing. Most assays using indicator organisms employ fish, invertebrates or algae (Little 1978). Little information is available on the use of bacteria as test organisms in assessing the toxic effects of pollutants.

The toxicity of selected arsenical compounds in short-term testing has been reported by ANDERSON & ABDELGHANI (1980). In the present study, the toxic effects of p-benzoquinone and hydroquinone are reported using Pseudomonas fluorescens.

Benzoquinone is capable of suppressing luminescense in certain bacteria and inhibiting succinate oxidase activity in Pseudomonas aeruginosa (WEBB 1966). It has also been investigated for its toxic properties on the algae, Selenastrum capricornutum (GIDDINGS 1979).

MATERIALS AND METHODS

Benzoquinone and hydroquinone were of reagent grade.

The test organism, P. fluorescens NRC 2137 was obtained from the National Research Council, Ottawa, Canada. The organism was grown in 1-L flasks containing 300 mL of nutrient broth (Difco) for 18 h at 28° C with shaking at 100 rpm.

Cells were aseptically harvested at 10,000 g for 10 min at 4° C, washed thrice in sterile phosphate buffer (150 mM, pH 7) and resuspended in the same buffer. The washed cell suspension was used in disc assays, survival studies and respirometric tests.

The effect of benzoquinone and hydroquinone on the growth of P. fluorescens was tested using No. 740E assay discs, 12.7 mm in diameter (Carl Schleicher and Schuell Co.). The discs were saturated with filter sterilized solutions of either benzoquinone or hydroquinone that were disolved in phosphate buffer. Petri plates containing nutrient agar were spread with 0.1 mL of washed cell suspension (equivalent to 100 µg/mL cell protein). Assay discs of various concentrations were placed on the solid agar surface.

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Plates were incubated at 28°C for 24 h, at which time the diameters of inhibition were measured (the diameter of inhibition includes the disc diameter).

One mL of the cell suspension was added to 100 mL aliquots of sterile minimal salts medium (TREVORS & BASARABA 1980) containing either 200 µg/mL of benzoquinone or hydroquinone. Test flasks were incubated at 28° C for 24 h with shaking. The number of viable cells were determined using the standard plate count procedure.

Oxygen consumption was determined at 28° C using a Warburg respirometer. Reaction vessels contained 2.7 mL of liquid. The sidearm recieved 0.5 mL of cell suspension which was tipped into the main well after an equilibration period of 10 min. Benzoquinone and hydroquinone were used at a final concentration of 10 µg/mL. Glucose was used as a standard substrate at 0.05% (w/v) concentration. Protein was measured by the method of LOWRY et al. (1951).

RESULTS AND DISCUSSION

The results in Table 1 show the effect of benzoquinone and hydroquinone on the growth of the test organism. The pseudomonad was strongly inhibited by benzoquinone at concentrations from 10 to 200 µg/mL. Hydroquinone was less toxic, causing inhibition at the 200 µg/mL level.

TABLE 1.

Inhibition of P. fluorescens growth by benzoquinone and hydroquinone as determined by the disc assay.

	zone of inhibition (mm)	
Concentration (µg/mL)	Benzoquinone	Hydroquinone
200	21 ± 4	14 ± 1
100	17 ± 2	12.7*
10	14 ± 1	12.7*

* 12.7-mm is diameter of disc, no inhibition.

After exposure to 200 µg/mL of benzoquinone or hydroquinone, the percent survival of resting cells of the test organism was 0.01 and 0.11 respectively, indicating the relative toxicity of these two compounds.

In respirometric trials, glucose was used as the standard substrate. In all tests the oxygen uptake was strongly inhibited by both compounds at a concentration of 10 µg/mL. The short-term toxic effect exerted by these compounds reduced the oxygen consumption to a level lower than that of endogenous consumption. Respirometric

tests normally carried out within a period of 1 or 2 h provide useful information. This is particularly true in the case of hydroquinone, where oxygen uptake is inhibited at 10 µg/mL, yet in the disc assay it is not toxic at the same concentration.

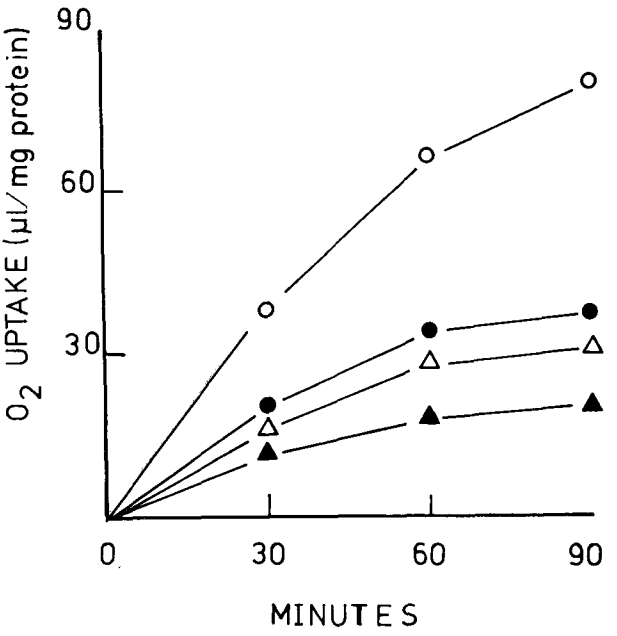

Figure 1. Inhibition of oxygen uptake by resting cells in the presence of benzoquinone (▲——▲), hydroquinone (△——△), control (o——o), endogenous (●——●).

The antibacterial activity of benzoquinone is believed to decrease with time, about 30% of its activity being lost in two weeks, and 50% in seven weeks (WEBB 1966). Nevertheless, short-term assays such as inhibition of growth, survival rates and respirometry provide useful information that can be obtained in a relatively short period of time. The disc assay, being similar to antibiotic disc assays provides rapid data on the range of concentrations that are toxic. Respirometry provides data on metabolic events that may not be observed in growth studies. However, it must be pointed out that short-term testing does not give microorganisms a chance to exert their full metabolic potential, such as adaptation of the test organism to the chemical being tested. Nevertheless, the short-term disc assay did show a distinct difference in the toxicity of benzoquinone and hydroquinone to the test organism, even though they are similar chemical compounds. Short-term bacterial bioassays could be a simple and accurate component of tier testing. Possibly their most useful aspect is that of quality control as well as the short time interval needed to conduct tests.

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