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Rapid Toxicity Testing Based on Yeast Respiratory Activity

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Rapid and economical techniques are needed to determine the effects of environmental contaminants. At present, the main methods to assess the impact of pollutants are based on chemical analysis of the samples. These procedures are time consuming and the reliability of a toxicity rating based upon their concentration is questionable, particularly in mixtures. The resultant toxic effect may be additive, synergistic, or antagonistic which at present can not be predicted from the toxicity data of individual chemicals.

Invertebrate and vertebrate exposures have been used over the last two decades in assessing acute and chronic toxicities. However, these tests are labor intensive and require several days to complete. Furthermore, results from whole organism toxicity tests are not easily interpretated in terms of mechanisms of toxicity.

An alternative to whole organism exposure is to determine toxic effects in monocellular systems, such as tissue cultures (Inmon et al. 1981), sperm cells (Dinnel et al. 1982), and bacteria (Curtis et al. 1982, Bulich et al. 1981). However, these tests also have disadvantages. Tissue and cell cultures require special facilities, and assessments may take from hours to days. While the bacterial assays are inexpensive and only take minutes to perform (Microtox bacterial assay system), they have not shown the same pattern of sensitivity to environmental samples as those elucidated in species such as fish (Qureshi 1982, Dutka and Kwan 1982).

Another approach for assessing toxicity is to monitor sensitive, nonspecific, subcellular target sites such as mitochondria (Ebina et al. 1980, Gopalaswamy and Aiyar 1984, Morita 1984, van Rossum et al. 1985, Wolf et al. 1982). This organelle is common to most eukaryotic cells of plants and animals, and is involved in a number of vital cellular processes. Changes in mitochondrial function (ie. state 3 and 4 respiration rates) which could indicate a toxic effect can be demonstrated readily after addition of a foreign substance (Figure 1).

In our initial assessments of various chemicals, rat liver mitochondria (RLM) were evaluated as a biological sensor of toxicity. However, the lability of mitochondria *in vitro* make them somewhat impractical for routine use as the sensor since they are very delicate and are easily damaged during isolation. Once extracted, RLM have a limited functional lifetime (24 to 48 hours) and are very sensitive to ions such as

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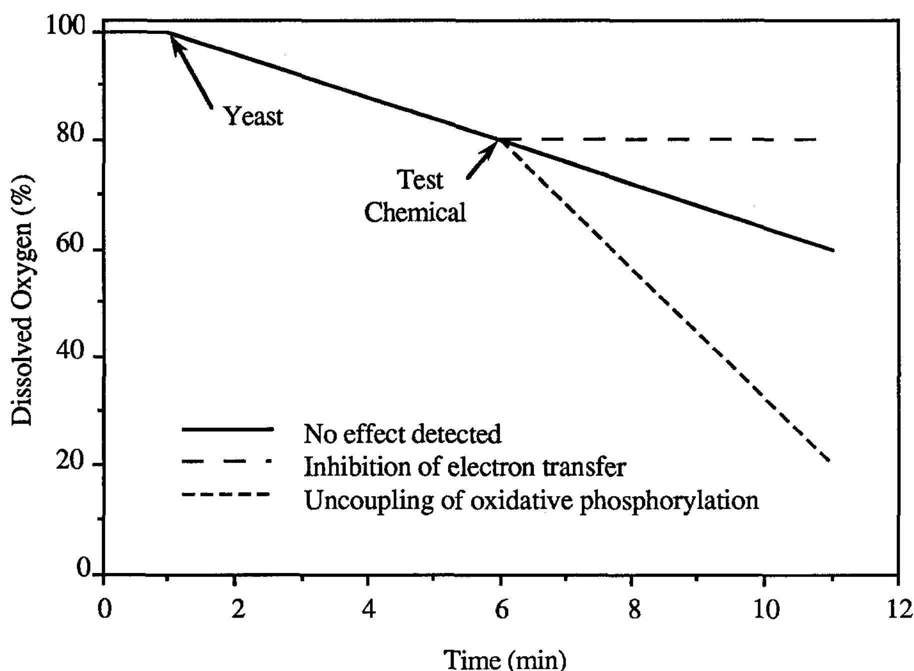

Figure 1. Ideal effects of chemical agents on yeast respiration.

calcium and iron. False toxicity assessments will result if these ions are present even though they are generally considered nontoxic. Because of these disadvantages, an alternative mitochondrial system, such as found in bakers yeast, was evaluated. Yeast are unicellular, more durable than isolated mitochondria, can be harvested without damage to the mitochondrial respiratory system, and are not affected when exposed to ions such as calcium and iron.

MATERIALS AND METHODS

The wild diploid strain of bakers yeast, Saccharomyces cerevisiae C 276 a/α (Pringle, Department of Biological Sciences, The University of Michigan) was used as the biological sensor and was grown in YM-1 medium (Pringle et al. 1975). After the medium was autoclaved-sterilized, 50 mL of 20% ethanol was aseptically added to each 450 mL aliquot of the medium for a carbon supplement.

The yeast cells were grown at 37.5°C and aerated at 130 rpm thoughout the incubation period. For assessment of respiration, cells were harvested by centrifugation at 1000 x g for five minutes. The cells were then resuspended in 20 mL yeast respiration assay medium (0.1 M potassium phosphate in 2% ethanol, final pH 5.8), and washed twice by centrifugation at 1000 x g for five minutes. The final pellet was resuspended in 3 - 4 mL assay medium and chilled at 0 - 4°C until needed. Fractions of the final cell suspensions were sonicated for 20 seconds to disperse clumps (Branson S-75 ultrasonic sonifier) and counted with a model Zb electronic particle counter (Coulter Electronics) equipped with a 100 μm diameter aperture tube (Pringle et al. 1975).

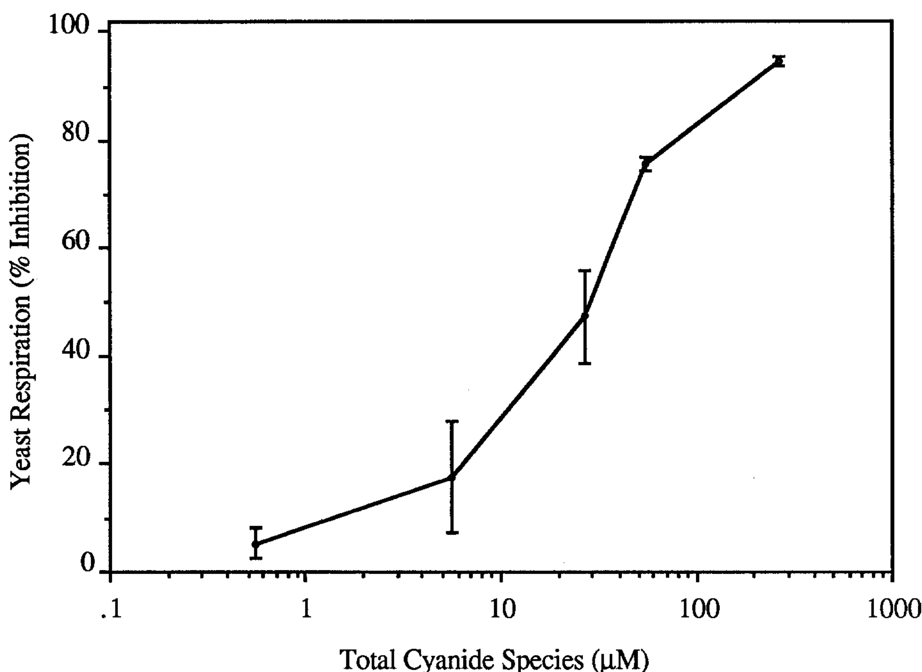

Figure 2. Effect of potassium cyanide on yeast respiration.

The oxygen electrode system (Yellow Springs Instruments, model #53) was calibrated with deionized water which had dissolved oxygen (DO) concentrations ranging from 0 - 100% air saturation as determined by Winkler titrations (American Public Health Association 1975). Zero DO was obtained by the addition of sodium sulfite (Hitchman 1978).

For each toxicity test, 1 x 10^7 yeast cells were injected into the closed assay system containing 2.5 mL air saturated yeast assay buffer at 30.0°C. After the initial respiration rate stablized, a volumetric quantity of a test sample was also injected. Since yeast cell membranes are impermeable to ADP, additions of ADP had no effect on the rate of respiration. Instead of determining state 3 and 4 rates and respiratory control ratios (RCR), percent change from control respiration rates were based on respiration before and after addition of the test solution.

Eleven of the test chemicals assessed in this study are listed in the United States Environmental Protection Agency (USEPA) list of water related priority pollutants (1979): isophorone, naphthalene, chlordane, n-nitrosodimethylamine, potassium cyanide, chloroform, diethyl phthalate, potassium dichromate, mercuric chloride, cupric chloride, and 2,4-dinitrophenol (2,4-DNP). Malathion was also included in this study as a representative of organophosphate pesticides. All solutions were prepared in deionized water, stored at 0 - 4°C, and brought to room temperature before use.

Table 1. EC_{50} values of yeast respiration for selected test chemicals.

Chemical	EC_{50} (mM) Stimulation	Inhibition
Potassium cyanide	*	30
2,4-Dinitrophenol	200	*
Mercuric chloride	100[a]	185
Cupric chloride	1470[a]	8000

*No effective concentration determined.
[a]Initial stimulated response.

RESULTS AND DISCUSSION

The concentration of DO in air saturated yeast assay media was found to be 7.02 mg $O_2 \cdot L^{-1}$. The electrode response in deionized water was linear through the range of DO tested (0 - 7.54 mg $O_2 \cdot L^{-1}$), with a sensitivity of 36.9 nAmpere$\cdot$L$\cdot$mg^{-1}. Forall assays: T = 30.0 ± 0.2°C, P = 750 ± 5 torr.

Malathion, isophorone, naphthalene, chlordane, diethyl phthalate, potassium dichromate, chloroform, and n-nitrosodimetnylamine (173 nM - 72.2 µM, 1.97 - 824 µM, 99.2 nM - 41.3 mM, 55.2 pM - 23.0 nM, 1.46 - 612 µM, 11.3 µM - 4.71 mM, 2.04 - 852 µM, and 3.94 µM - 1.64 mM, respectively) induced no detected effect on the rate of yeast respiration.

Cyanide ion (552 nM - 271 mM) inhibited over-all respiration, which was characteristic of electron transfer inhibition (Figure 2), and 2,4-dinitrophenol (948 nM - 396 mM) stimulated respiration, which was indicative of the uncoupling of oxidative phosphorylation from electron transfer.

Both mercuric and cupric ions (1.92 - 802 µM and 30.0 µM - 12.5 mM, respectively) caused an initial stimulation followed by an inhibition of respiration. This initial effect only persisted for a few minutes before the respiration rates decreased to values less than before addition of the test solutions. Similar effects of this type have been reported for Ag^{+}, Hg^{2+}, and Cu^{2+} on respiration in molds (Webb 1963), and for Zn^{2+} on respiration in isolated mitochondria (Kleiner 1974). These authors felt that an uncoupling of oxidative phosphorylation was followed by an inhibition of electron transfer. If this was the case, then this would help explain the variance that was generated in the initial stimulated respiration rates: inhibition of electron transfer caused a cessation of respiration which partially masked a stimulation produced by an uncoupling process. Concentrations of the test chemicals determined to produce a 50% effect (EC_{50}) on yeast respiration are presented in Table 1. Compounds tested that indicated no effect on yeast respiration are not included.

A series of experiments were also carried out to evaluate the effect of "harvest" and "viability" of yeast on their respiration rate. Yeast cells sampled at different periods along the growth curve had very similar respiration rates for a set number of cells. Therefore, it was not necessary to harvest them at a specific point along the growth curve. Time-extended viability was also tested for up to 12 days after initially harvesting the yeast. No major change in respiration throughout this time was detected. The cells were stored at 0 - 4°C when not being assayed. On the twelfth day after harvest, the cells were evaluated with 2,4-DNP and with mercuric chloride. Similar results to those with freshly harvested yeast were observed, which indicated a rather long stability for the suspensions.

A dissolved oxygen electrode is used in this system to assess toxicity by monitoring changes in the respiratory rate of intact yeast cells. The described test is one of the most rapid and inexpensive methods ever suggested for screening contaminants in environmental samples. Although the yeast are generally not as sensitive as isolated RLM to indicate a toxic response, their prepartation, storage life, and physical durability make them more practical for a toxicity sensor of this type.

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