# A Rapid Respiratory Toxicity Test Using *Caenorhabditis elegans* with an Oxygen Electrode System

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**We describe a novel approach to evaluating the respiratory toxicity of chemicals in the free-living nematode *Caenorhabditis elegans*. Using DOX-96KT, a general purpose, multi-channel dissolved oxygen (DO) measuring system, we measured the DO concentration in culture media containing *C. elegans* exposed to chemicals to assay for respiratory toxicity. The current value, which is an index of the dissolved oxygen concentration in culture media, was measured every 10 sec for 30 min at 24°C. We focused on the respiration levels of the exposed worms between 500 and 1800 sec. This method produces results that are similar to the computer tracking system measuring behavioral toxicity. Since it can do multiple dilution series tests at a given time, it is useful for concentration-activity correlation studies. This novel technique is not only an alternative to the computer tracking system for measuring behavioral toxicity but also a rapid sublethal toxicity test for chemical hazard assessment.**

**Key words** —— *Caenorhabditis elegans*, respiratory toxicity, oxygen electrode, behavioral toxicity

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## INTRODUCTION

In recent years there has been increasing need for a rapid and automated assay system to measure the toxicity of chemicals in lower concentrations. Several studies have been conducted on chemical hazard assessment using *Caenorhabditis elegans* (*C. elegans*), a free-living nematode with a completely mapped genome that has already become a commonly used laboratory model for molecular and cellular biology.[1,2] Toxicant evaluation has several endpoints, with lethality and reproduction often used.[3,4] Several other endpoints have been assessed using *C. elegans* such as the behavioral effects of toxicants using a computer tracking system.[5,6] In designing an automated high output assay, simplicity is the key, with the detection method requiring as few steps as possible. With this in mind, we used an oxygen electrode sensor to monitor the respiratory activity of *C. elegans* instead of monitoring their movements. The dissolved oxygen (DO) concentration in culture media is detected and monitored as an electrical current to indicate the respiratory activity of the worms. When worms' activity is higher, the DO concentration is lower. Conversely, when the organisms are injured or killed, the DO concentration is expected to increase. A general purpose, multi-channel dissolved oxygen measuring system appears to be an ideal instrument for such testing, and can be applied to rapid antibiotic screening,[7] among other uses.

In this letter, we demonstrate the use of a rapid and automated *in vivo* assay system for measuring respiratory toxicity in *C. elegans* using the oxygen sensor together with the multi-electrode measuring system. This system is dubbed the rapid respiratory toxicity test.

## MATERIALS AND METHODS

Wild type *C. elegans* were grown and maintained as described by Brenner,[8] and routinely cultured at 20°C on glass, acid-washed petri plates containing NGM agar and Escherichia coli as food source.[8]

Tests were conducted with age-synchronized worms.[6] Synchronized juvenile worms (first stage) were then individually transferred to a NGM agar plate containing the chemicals and a lawn of *E. coli*. The set-ups were then incubated at 20°C for 46 hr, and then the worms were rinsed with fresh M9 buffer. The washed worms were transferred to a 96-well

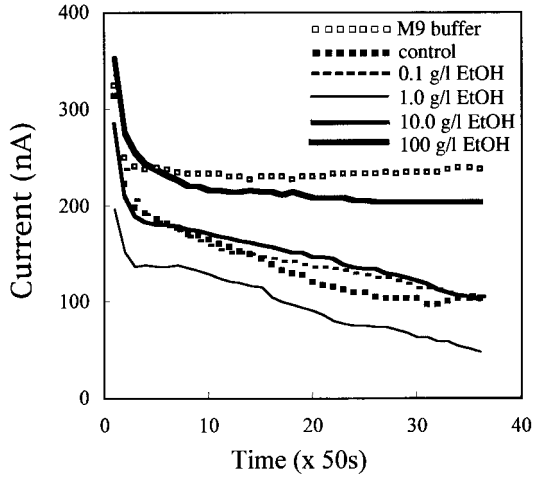


**Fig. 1-1.** Oxygen Consumption Profile in Culture Media with 150 Worms

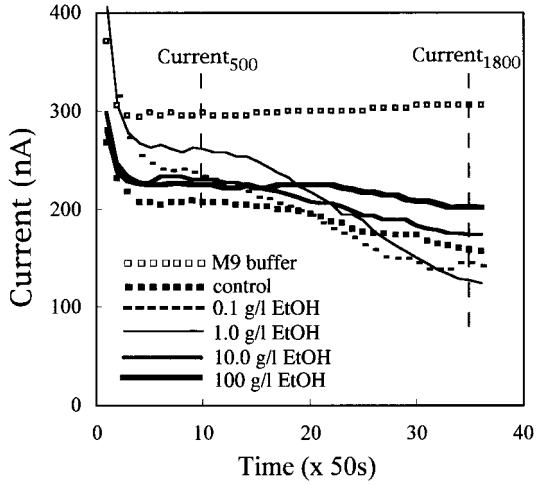


**Fig. 1-2.** Oxygen Consumption Profile in Culture Media with 200 Worms

microplate equipped with a disposable electrode plate inserted from the surface of each well. M9 buffer was placed in each well. The exposed worms were then loaded to a final concentration of approximately 150 or 200 per 200 $\mu$l of M9 buffer. The current value, which is an index of dissolved oxygen concentration, was measured every 10 sec for 30 min at 24°C using DOX-96KT (Daikin Environmental Laboratory, Ltd.), a general purpose, multi-channel dissolved oxygen measuring system.

## RESULTS AND DISCUSSION

Initially, the respiratory activity of *C. elegans* was examined on exposure plates containing four

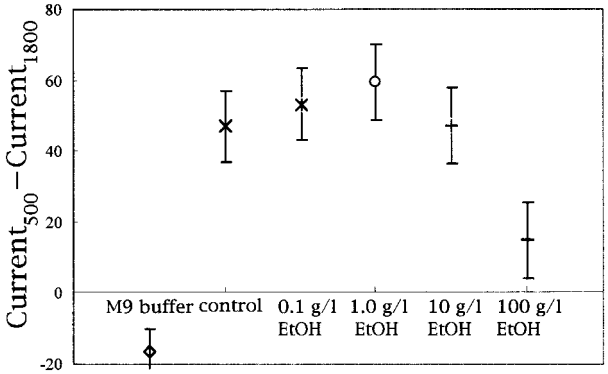


**Fig. 2-1.** Influence of EtOH on Respiration Levels of *C. elegans* (150 worms, $n = 11$)

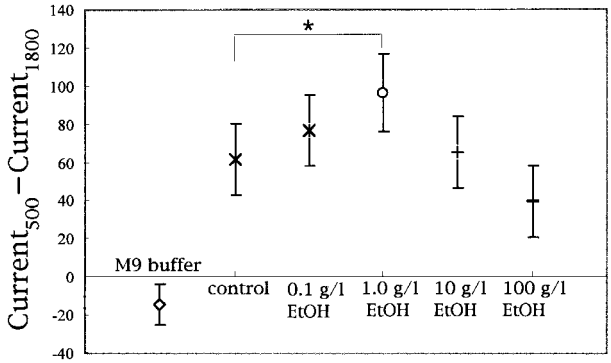


**Fig. 2-2.** Influence of EtOH on Respiration Levels of *C. elegans* (200 worms, $n = 6$) *) $p < 0.05$ statistically significant.

concentrations (0.1 g/l, 1.0 g/l, 10 g/l, and 100 g/l) of ethanol. The electric current was recorded every 10 sec for 30 min under an applied constant voltage of –600 mV.[9] Typical oxygen consumption profiles of 150 and 200 worms per well are shown in Figs. 1-1 and 1-2, respectively. The term "respiration level" as used in the figures refers to the difference obtained by subtracting the current at 1800 sec from the current at 500 sec. Based on the respiration levels of 150 and 200 worms, exposure to 1.0 g/l ethanol led to an increase in the respiration levels of *C. elegans* (Figs. 2-1 and 2-2). These results agree with our feeding behavioral test using *C. elegans* exposed to the same concentration (Fig. 3). However, when the organisms were exposed to 100 g/l ethanol, the respiration levels decreased compared to the untreated control. Generally, the rapid respiratory toxicity test, which measures respiration levels, produces results that are similar to the computer tracking system.[5] Figure 4 shows data using the other compounds examined; dimethyl sulfoxide (DMSO), bisphenol A (BPA) and nonylphenol (NP). In the case

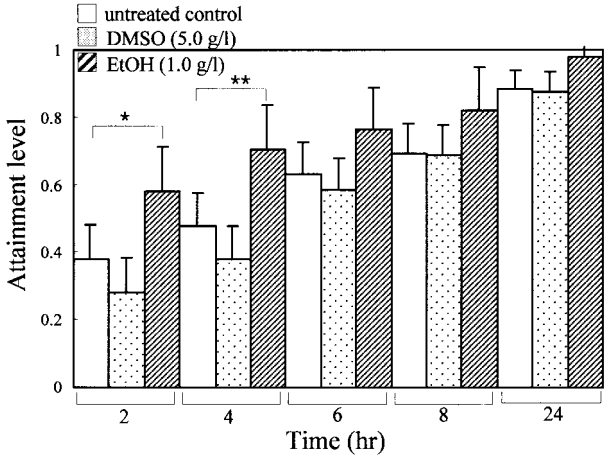


**Fig. 3.** Influence of Solvent on Attainment Levels of *C. elegans*
*) $p < 0.05$, **) $p < 0.01$ statistically significant.

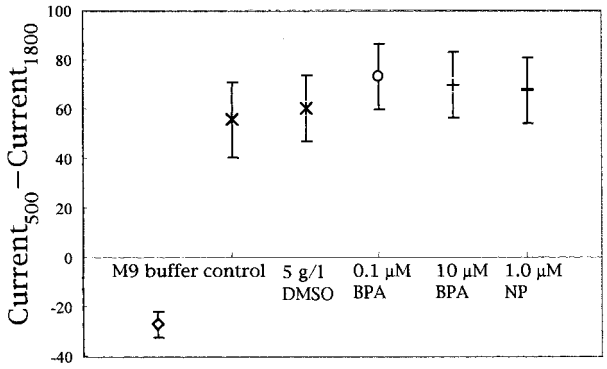


**Fig. 4.** Influence of Chemicals on Respiration Levels of *C. elegans*
(200 worms, $n = 6$)

of BPA, the respiratory toxicity test results were different from those obtained during the feeding behavior test. The attainment levels of *C. elegans* exposed to 0.1 $\mu$M or 10 $\mu$M BPA decreased significantly ($p < 0.05$) compared to the untreated control.[10] We have no definite explanation for the difference, but it may be that BPA affects not movement but the sense organs.

We demonstrated here a rapid and automated in vivo assay system using *C. elegans* coupled to an oxygen sensor with a multi-electrode measuring system (DOX-96KT). This system is useful for concentration-activity correlation tests, such that multiple dilution series can be done at one time. It is also affordable, while utilizing a disposable sensor, commercially available 96-well plates, and a multichannel measuring system. The system has been used practically for estimation of bacterial cell count as well as antibiotics screening.[7,11] When the testing conditions and analysis method[9] are optimized by further experiments, this novel technique can not only be an alternative to the computer tracking system for measuring behavioral toxicity but also a rapid sublethal toxicity test for chemical hazard assessment.

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