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Novel Temperature Control Apparatus for Whole Effluent Toxicity Tests

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Whole effluent toxicity (WET) tests conducted for National Pollutant Discharge Elimination System permits which follow the United States Environmental Protection Agency (USEPA) methods must adhere to a stringent temperature criteria. Each of the methods in Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms (USEPA 2002a) recommend that temperature be maintained at 25 ± 1°C and require that the test temperature does not deviate by more than 3°C. The test temperatures required in Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (USEPA 2002b) vary depending on test species and objectives of the test. However, it is recommended that test temperatures be maintained ±1°C of the selected test temperature. The apparatus for maintaining temperature may be "circulating water baths, heat exchangers, or environmental chambers" (EPA 2002a). The most common temperature-control apparatus used is an environmental chamber (*M. Matthews, North Carolina Department of Environment and Natural Resources, personal communication*). The apparatus described here is a simple, inexpensive temperature control system using a water bath and waterbed heater. It can be used to control test-chamber temperature during performance of the cladoceran (*Ceriodaphnia dubia*) survival and reproduction test (hereinafter referred to as the *Ceriodaphnia* test) and the fathead minnow (*Pimephales promelas*) larval survival and growth test (hereinafter referred to as the fathead minnow test) as per USEPA methods (USEPA 2002a) and may also be applicable to other experimental systems that require temperature control (e.g., sediment or marine toxicity tests (USEPA 2002c, 1994)).

MATERIALS AND METHODS

The temperature control system consisted of a waterbed heater and plastic photo tray (20 cm x 24 cm x 8 cm; Cesco-lite, Photoquip Inc., USA) containing water (Fig. 1). For a *Ceriodaphnia* WET test, a plexiglass holding tray containing 60 test beakers (30-mL plastic disposable) was placed into a photo tray. For a fathead minnow test, a minimum of 24, 600-mL glass beakers were placed into a photo tray (one tray will hold 36, 600-mL beakers). The water level in the photo tray

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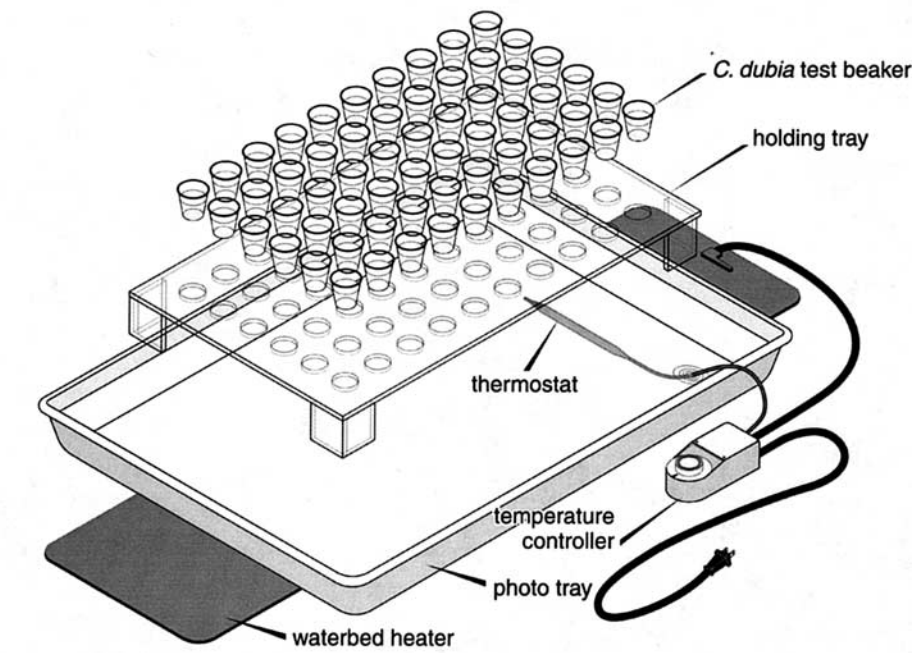

Figure 1. Diagram of temperature control device for *Ceriodaphnia* test using waterbed heater.

was as high as practical for the test vessel and was replenished daily. To assess system performance, the temperature of the water in the photo tray (water bath) was controlled by placing the thermostat of the waterbed heater into the photo tray and adjusting the temperature controller.

The temperature control systems were transported to an out-of-state college laboratory, assembled, and used for WET tests with *Ceriodaphnia* and fathead minnows. The only temperature control available was the building's heating and cooling system. Five *Ceriodaphnia* tests (i.e., 10 photo trays) and 5 fathead minnow tests (i.e., 8 photo trays) were conducted during August and October. The water bath and beaker temperatures were measured in each photo tray approximately once per day for the 6- or 7-day test period using a hand held thermistor. The calibration of the thermistor was checked with a certified glass thermometer prior to use. The room temperature was not measured but was maintained at levels comfortable for laboratory work (approximately 21°C). Temperature measurements were obtained for 73 comparisons of *Ceriodaphnia* test beakers and corresponding water bath and 57 comparisons of fathead minnow.

In addition to the WET tests, a laboratory experiment was conducted at Oak Ridge National Laboratory using the *Ceriodaphnia* test to evaluate (1) the variability of

water temperature in beakers at various locations in one holding tray and (2) the ability of the waterbed heater to maintain water temperature in a beaker at 25 ± 1°C when the air temperature quickly dropped. One temperature control system with 60 *Ceriodaphnia* test beakers containing distilled water was placed in a temperature controlled chamber. Over the course of 90 min, the temperature was lowered in 2 degree increments from 25 to 9°C. The temperature controller of the waterbed heater was not adjusted. After the temperature of the chamber had stabilized, the temperature was measured in the water bath and in 11 test beakers positioned diagonally across the holding tray.

RESULTS AND DISCUSSION

During the WET tests conducted at room temperature, mean temperature of the water baths was 25.5 ± 0.6°C and 25.0 ± 0.9°C for the *Ceriodaphnia* and fathead minnow, tests respectively. Mean temperature of the effluent in the beakers was 25.0 ± 0.6°C and 24.9 ± 0.8°C for the *Ceriodaphnia* and fathead minnow tests, respectively. The mean absolute difference between the temperature of the water in each beaker and 25°C was 0.5 ± 0.4°C for the *Ceriodaphnia* test and 0.6 ± 0.6°C for the fathead minnow test. Of the 73 comparisons for the *Ceriodaphnia* test, only four beaker temperatures were outside of 25 ± 1°C (23.1, 26.1, 23.9, and 23.3°C). Of the 57 comparisons for the fathead minnow test, 15 beaker temperatures were outside of 25 ± 1°C (range: 23.9 to 27.1°C).

Results of the laboratory experiment with *Ceriodaphnia* in an environmental chamber are summarized in Figure 2. The temperature control system maintained both the water bath and beaker temperature at needed levels down to an air temperature of 8°C. This demonstrates that the system will maintain beaker temperature should there be a rapid decrease in air temperature during the test period. Beaker temperature exceeded 26°C at ambient air temperatures of 18.8 °C and 16.5 °C. Because the beaker temperature was maintained at 25 ± 1°C at lower (14.6°C) and higher (20.2°C) air temperatures, it appears that the beaker temperature could be maintained at 25 ± 1°C with minor adjustments to the waterbed temperature controller. The variability from beaker to beaker tended to be higher at the lower air temperatures, but it did not exceed 25 ± 1°C.

The greatest utility of this temperature control system for WET tests or for other compliance-related testing will be for short-term use where other alternatives are not available and flexibility is important. The Toxicology Laboratory at Oak Ridge National Laboratory has used this system to conduct quarterly WET tests at an out-of-state facility. The system is easy to assemble and inexpensive.

Temperatures were maintained within the prescribed limits (25 ± 1°) for both the *Ceriodaphnia* and fathead minnow tests. The system did slightly less well at maintaining temperature using the fathead minnow test beakers, probably due to

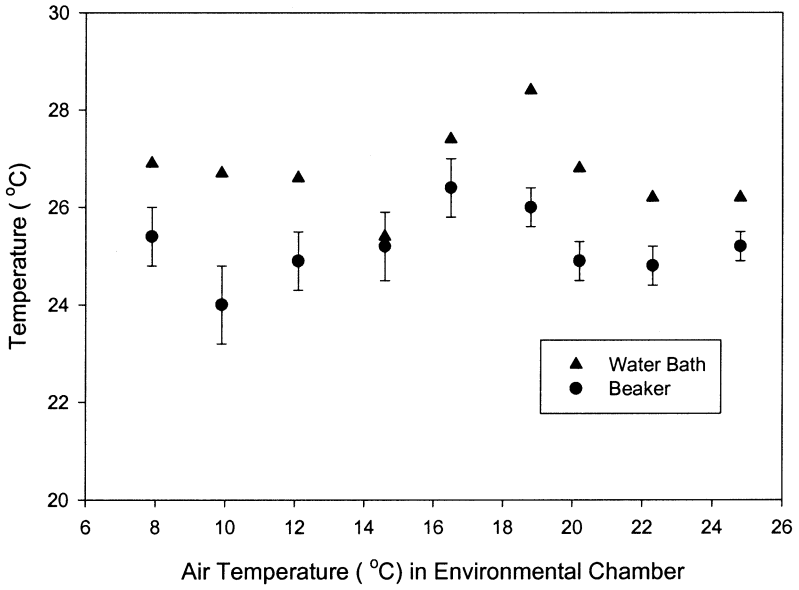

Figure 2. A comparison of water bath temperature and beaker temperature in the environmental chamber. Chamber temperature was decreased from 25 to 9°C over the course of 90 min. Error bars represent standard deviation.

the lack of water circulation around the beakers. Adding an air line or other device to circulate the water within the photo tray would probably improve the temperature control. Daily vigilance is very important in maintaining temperature using this system: the temperature of the water bath will drift, water level within the photo tray must be maintained at the maximum level, and each temperature controller responded differently to adjustments. The fact that temperature will vary across a *Ceriodaphnia* holding tray (and probably among fathead minnow beakers in one water bath) makes it critical that test beakers be randomized.

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