



## **Tier II Antimicrobial Efficacy Testing of AgriTec**

**Performed by NSF Microbiology Laboratory for The Toxicology Group LLC**

**Client:** Earth Science Laboratories, Inc.  
**Product:** AgriTec  
**Date of Experimentation:** July 31, 2002  
**Test Organism:** *Klebsiella terrigena*  
**ATCC Strain:** 33257  
**Copper Concentration:** 0.65, 1.3, 13, 130 ppm

### **BACKGROUND**

The Client has contracted the Toxicology Group, LLC to perform an initial assessment of the fungicidal properties of their product. *Klebsiella terrigena* ATCC 33257 was selected as a challenge organism. *K. terrigena* is an enteric bacteria commonly used as a surrogate test organism for *K. pneumoniae*. *K. pneumoniae* is a common coliform and was selected as the challenge organism to represent the coliform group in the USEPA Guide Standard and Protocol for Testing Microbiological Water Purifiers.

### **METHODOLOGY**

#### **A. Stock Culture Preparations:**

The bacterial challenge strain was reconstituted in nutrient broth and passed 3 times. All culture tubes were incubated at 35°C for 24 hours. Twenty-four hours prior to testing, the bacterial challenge organism was inoculated into 500 ml of nutrient broth and incubated at 35°C. After incubation, the culture was centrifuged at 2500 rpm for 10 minutes. The supernatant was discarded and the remaining microbial pellet was washed three times in sterile buffered deionized water (SBDW). After the third centrifugation step, the pellet was resuspended in 50

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mL of SBDW. An approximate cell density of the suspension was obtained either via direct staining with acridine orange or plating on growth media.

**B. Protocol:**

Five 500 mL sterile Erlenmeyer flasks containing 300 mL of sterile tapwater were spiked with the challenge organism to achieve a minimum final concentration of  $1 \times 10^6$  CFU/mL. The following AgriTec concentrations (as a function of active copper) were amended to individual test flasks: 0.65 ppm, 1.3 ppm, 13 ppm, 130 ppm. An inoculum control containing no AgriTec was also setup. All flasks were placed on a rotary shaker set at 50 rpm at 25°C. 1 mL from each flask was aseptically removed at the following time points: 0 hours, 10 minutes, 1 hour, 3 hours, and 24 hours and transferred to 1 mL of a neutralizer solution consisting of lecithin, Tween 80 and phosphate buffer. Serial dilutions were carried out in SBDW. 0.1 or 1 mL aliquots were pour-plated using Standard Plate Count Agar (SPCA). The plates were incubated at 35°C for 24 hours prior to enumeration.

**RESULTS**

**Table 1.** Survivability of *K. terrigena*. Exposure time is provided in minutes. Microorganism concentrations at the copper concentrations are provided in Colony Forming Units per milliliter (CFU/mL).

| Exposure Time | 0 ppm Copper       | 0.65 ppm Copper    | 1.3 ppm Copper     | 13 ppm Copper      | 130 ppm Copper     |
|---------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| <b>0</b>      | $2.12 \times 10^7$ | $1.32 \times 10^6$ | $1.38 \times 10^7$ | $1.61 \times 10^7$ | $1.54 \times 10^7$ |
| <b>10</b>     | $1.41 \times 10^7$ | $7.80 \times 10^6$ | $5.10 \times 10^6$ | $2.57 \times 10^6$ | $1.00 \times 10^1$ |
| <b>60</b>     | $1.73 \times 10^7$ | $7.30 \times 10^5$ | $1.42 \times 10^6$ | $1.34 \times 10^4$ | $1.00 \times 10^1$ |
| <b>180</b>    | $7.00 \times 10^6$ | $1.60 \times 10^5$ | $2.60 \times 10^5$ | $1.80 \times 10^2$ | $1.00 \times 10^1$ |
| <b>1440</b>   | $1.12 \times 10^5$ | $1.00 \times 10^1$ | $1.40 \times 10^3$ | $1.00 \times 10^1$ | $1.00 \times 10^1$ |

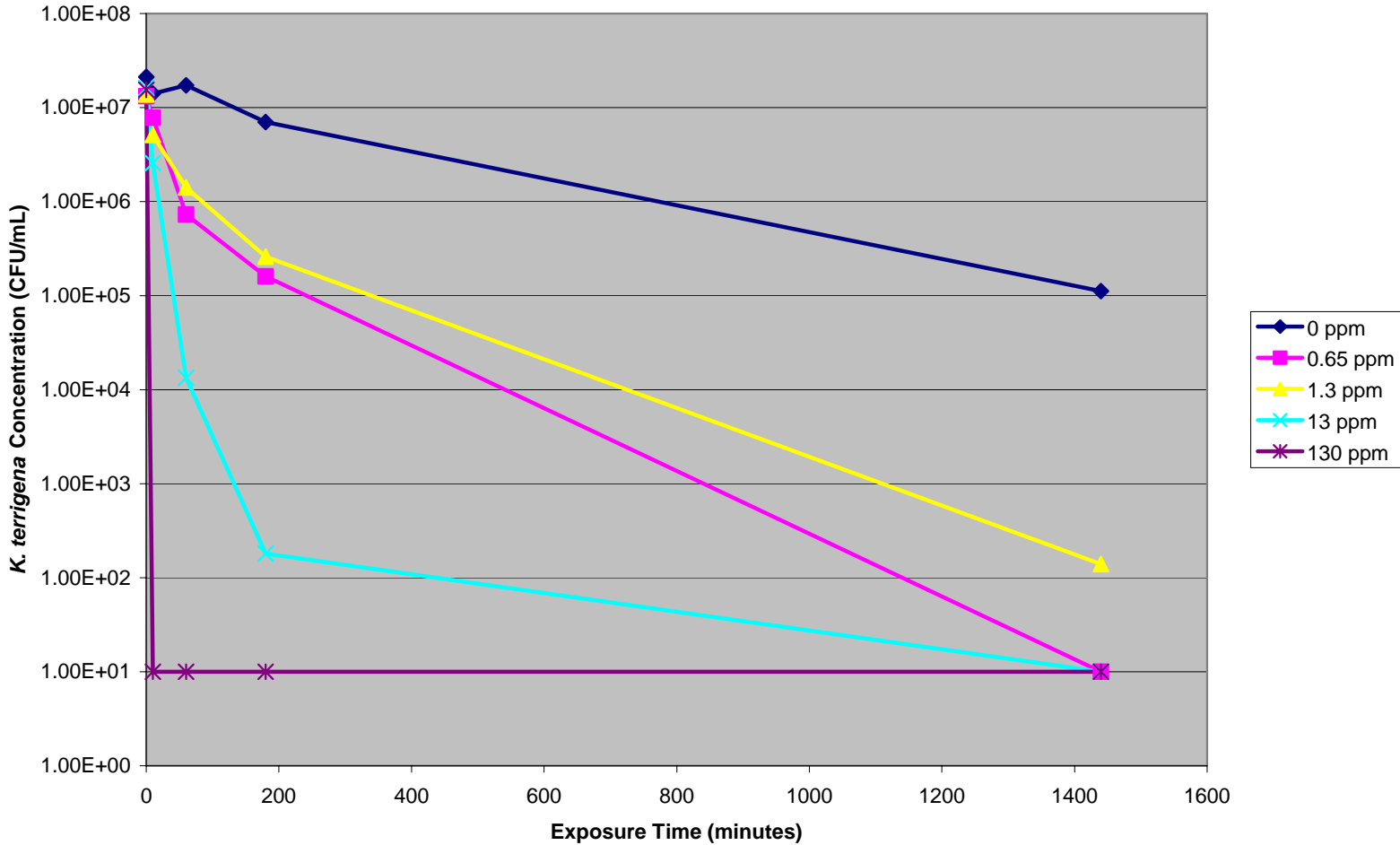
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**Figure 1.** Survival of *K. terrigena* at varying concentrations of copper. The limit of detection is 10 CFU/mL.

***Klebsiella terrigena* Survival at Varying Concentrations of Earthtec**



**SUMMARY**

As is evident, the 130 ppm concentration of AgriTec resulted in the highest rate of inactivation of *K. terrigena*. 13 ppm and 1.3 ppm were also effective in inactivating the challenge organism. 0.8 and 11 ppm exhibited no enhanced inactivation of the challenge organism when compared to the control group.

Laboratory Supervisor: RDJ Date: 5-14-03

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