



# Initial 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 18, 2002  
**Test Organism:** *Escherichia coli*  
**ATCC Strain:** 11229  
**Copper Concentrations:** 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. *Escherichia coli* ATCC 11229 was selected as a challenge organism. *E. coli* is a common gram negative bacteria often used as a indicator of fecal contamination. This particular strain is specified for water testing.

## 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 mL of SBDW. An approximate cell density of the suspension was obtained via direct staining of an aliquot of the challenge suspension with acridine orange and visualization at 1000X using fluorescent microscopy.

### B. Protocol:

Five flasks (one control and four experimental) containing 300 mL of tapwater were spiked with the challenge organism to achieve a minimum final concentration of  $1 \times 10^4$  CFU/mL. The following



dilutions of AgriTec were added to the appropriate flasks to achieve target copper levels of 130 ppm, 13 ppm, 1.3 ppm and 0.65 ppm:

- 130 ppm – 78 mL aliquot of a 1:100 dilution of AgriTec was added
- 13 ppm – 7.8 mL aliquot of a 1:100 dilution of AgriTec was added
- 1.3 ppm – 0.78 mL aliquot of a 1:100 dilution of AgriTec was added
- 0.65 ppm - 0.39 mL aliquot of a 1:100 dilution of AgriTec was added

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

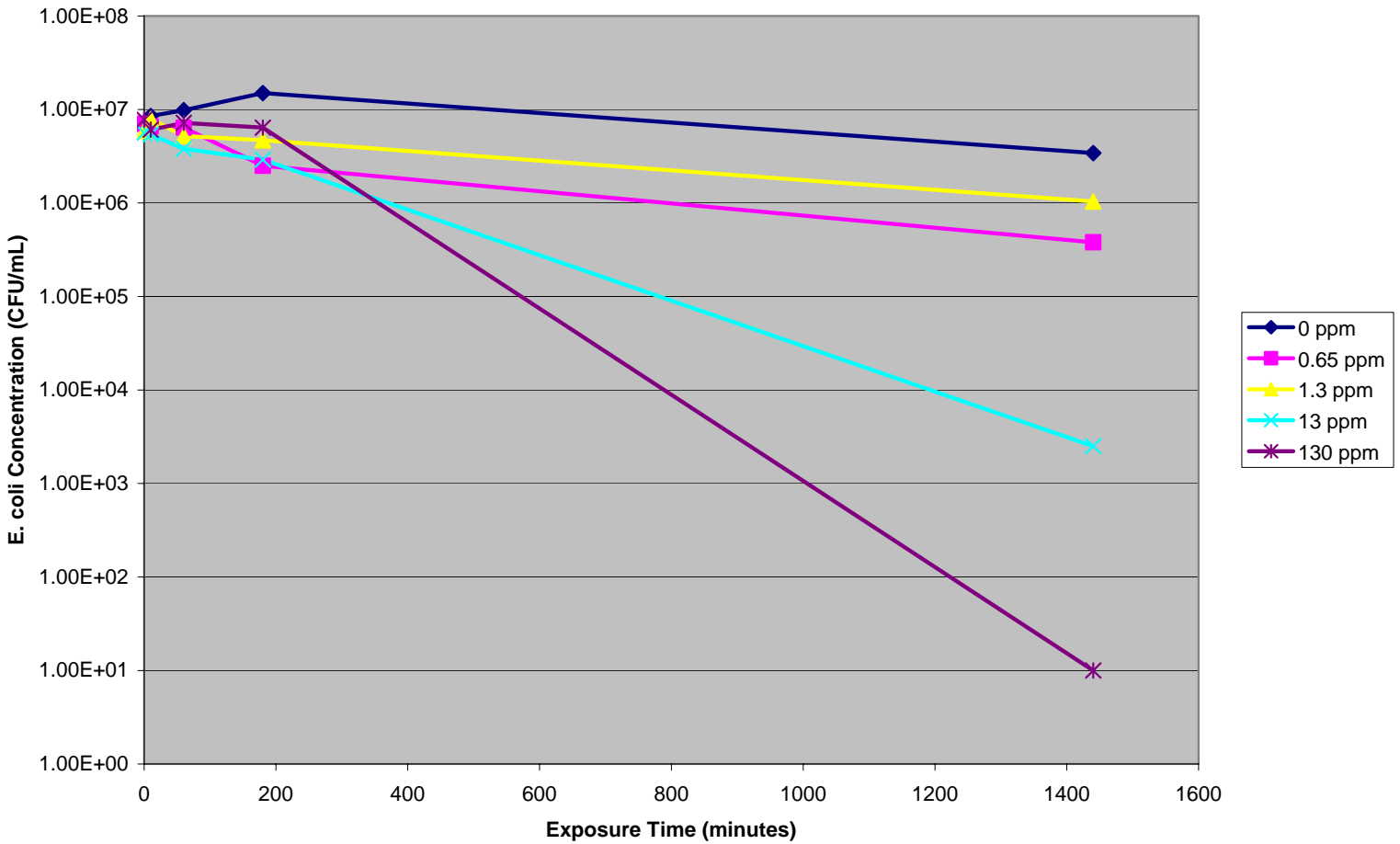
**Table 1.** Survivability of *E. coli*. Exposure time is provided in minutes. Microorganism concentrations are provided in Colony Forming Units per milliliter (CFU/mL).

Exposure Time	0 ppm Copper	0.65 ppm Copper	1.3 ppm copper	13 ppm Copper	130 ppm Copper
<b>0</b>	$6.30 \times 10^6$	$7.00 \times 10^6$	$5.90 \times 10^6$	$5.60 \times 10^6$	$7.80 \times 10^6$
<b>10</b>	$8.50 \times 10^6$	$6.60 \times 10^6$	$7.90 \times 10^6$	$5.40 \times 10^6$	$6.10 \times 10^6$
<b>60</b>	$9.80 \times 10^6$	$6.40 \times 10^6$	$5.20 \times 10^6$	$3.80 \times 10^6$	$7.20 \times 10^6$
<b>180</b>	$1.50 \times 10^7$	$2.50 \times 10^6$	$4.70 \times 10^6$	$2.90 \times 10^6$	$6.40 \times 10^6$
<b>1440</b>	$3.42 \times 10^6$	$3.80 \times 10^5$	$1.04 \times 10^6$	$2.50 \times 10^3$	$<.00 \times 10^1$



**Figure 1.** Survival of *E. coli* at 0.65, 1.3, 13 and 130 ppm of copper. The limit of detection is 10 CFU/mL.

**E. coli Survival at Varying Concentrations of Copper**



Laboratory Supervisor: RDJ Date: 5-14-03