

## Efficacy against *Legionella pneumophila* bacteria (free-swimming and in slime layers)

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**Important Note:** There is no EPA approved protocol for testing the efficacy of biocides against *Legionella pneumophila* in order to support an EPA label claim. However, the efficacy testing reported in this document was performed at the world-renowned Special Pathogens Laboratory of VA Medical Center in Pittsburgh, PA which specializes in the identification and quantification of Legionella.

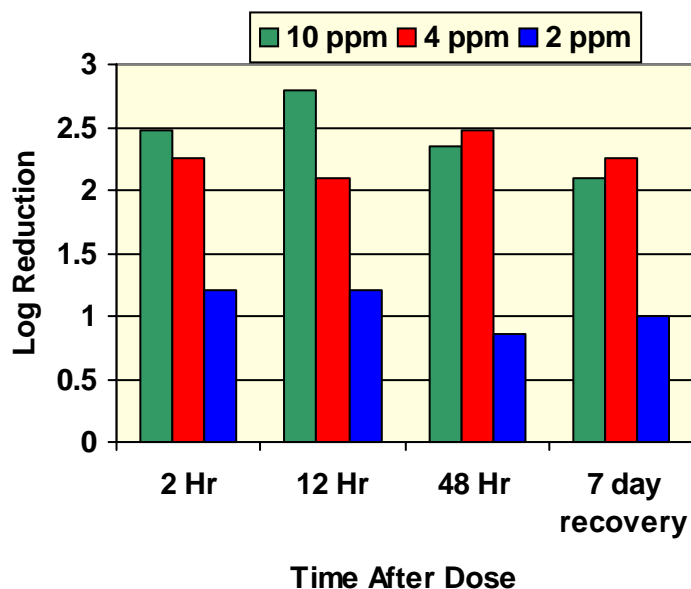
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### Summary of Laboratory Tests

- Stabilized bromine is shown to inactivate *Legionella pneumophila* bacteria cultured from an authentic cooling water.
- Stabilized bromine shows laboratory efficacy against both free-swimming and slime layer *Legionella pneumophila* bacteria.
- A dose of 4-10 ppm as Br<sub>2</sub> provides a 4-log reduction in the number of free-swimming (planktonic) bacteria. Even after removal of the active ingredient (recovery period), this reduction was maintained over the next 7 days.
- A dose of 4 ppm as Br<sub>2</sub> provides over a 2-log reduction in the number of slime layer (sessile) bacteria. A dose of 10 ppm as Br<sub>2</sub> provides a 2.7 log reduction. Even after removal of the active ingredient (recovery period), this reduction was maintained over the next 7 days.

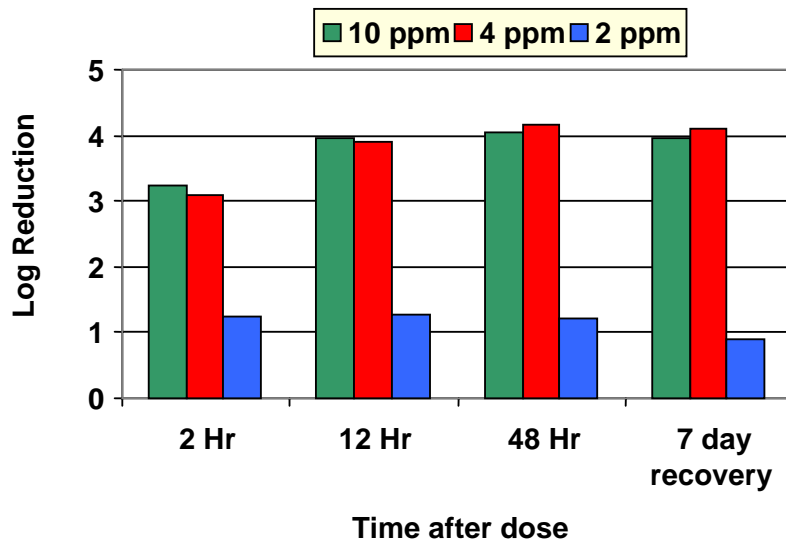
### Stabilized bromine vs. slime layer (sessile) *Legionella pneumophila*

The initial bacterial counts were ~ 1 x 10<sup>4</sup> CFU/cm<sup>2</sup> for the 10 ppm and 4 ppm concentrations and ~ 3 x 10<sup>5</sup> CFU/cm<sup>2</sup> for the 2 ppm concentration.



## Stabilized Bromine vs. free-swimming (planktonic) *Legionella pneumophila*

The initial bacterial counts were  $\sim 1 \times 10^6$  CFU/mL for all three concentrations.



Note that the ability of stabilized bromine to control the growth of, or inactivate *Legionella pneumophila* in operating cooling water towers exposed to ultraviolet light, organic material, other microbial contamination, and aeration was not the purpose of this study.

### **Experimental Conditions:**

The system consisted of three parallel transparent PVC sampling pipes. These pipes were used for collection of slime layer bacterial samples; one as control pipe, one for maximum recommended biocide concentration and the third for minimum concentration. Stabilized bromine was dosed to the system and the concentration adjusted to achieve the desired level during the first hour of exposure. The biocide challenge was divided into three phases – a 14-day culturing period; a 48-hour disinfection period; chemical neutralization of the bromine with a 7-day recovery period.

### **Microbiological Procedures:**

The source of naturally grown *Legionella pneumophila* was sediment and associated water obtained from the recirculating hot water cooling water basin of a hospital in the Pittsburgh, PA area. The sediment was collected from filter cartridges suspended in the water system for about two months. The sediment and filter water were harvested and the presence of *Legionella pneumophila* serogroups 1 and 6 were confirmed using direct fluorescent antibody staining (DFA) techniques.

The inoculum for the biocide challenge experiments consisted of dechlorinated tap water, *Legionella pneumophila* stock solution, and nutrient supplemental solution. The inoculum was cultured by incubation at 37 °C for 14 days prior to the tests. It was introduced to the model water system along with additional dechlorinated tap water. This mixture was recirculated throughout the system intermittently at 3.2 gpm for 14 days to produce a consistent slime layer bacteria population.

Samples of slime layer bacteria were collected at (i) the end of the 2-week culture period, (ii) during the biocide challenge period (0,2,12 and 48 hours), and (iii) during the 7 day recovery period . The samples were taken by swabbing the inner surface of a pre-measured section (length = 17/32") of the sampling pipe using a sterile swab. The swabs were vortexed for 1 minute in 5 mL of sterilized DI water with 0.1 mL neutralizer before plating. Free swimming bacteria samples were taken at the same time periods as the slime layer bacteria samples.

**Recovery Period:**

After the biocide challenge, the recirculation loops were drained and refilled with heat-sterilized tap water and samples after 7 days for *Legionella pneumophila* enumeration.

*ENVIRO TECH CHEMICALS, INC. has several patents pending in the field of stabilized bromine products and processes. One such product, scheduled for release in spring of 2005, is called BromMax, and is a 23%(as Br<sub>2</sub>) by weight active stabilized bromine. It has 68% greater activity than Nalco's Sta-br-ex, and 48% greater than Albemarle's StaBrom 909 products.*