Photo courtesy Kathryn Hedges.

**ALAN E. LEVITON STUDENT RESEARCH AWARD REPORT**

# *Copper Exposure Damages Both Dopaminergic and GABAnergicNeurons in the Nematode Caenorhabditis elegans*

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Kathryn Hedges is one of two recipients of the 2008 AAAS, Pacific Division Alan E. Leviton Student Research Awards. Bruce A. O'Gara was her advisor.

My research explored the hypothesis that copper selectively damages neurons in the nematode, Caenorhabditis elegans. Using strains expressing green fluorescent protein (GFP) in either specific neuron types or in the excretory cell allowed me to observe any damage after copper exposure. My results clearly found that exposure to CuSO4 damaged particular neurons in a time and concentration dependent manner compared to the excretory cell, and induced paralysis at sublethal concentrations.

The strain of C. elegans with excretory cell GFP I used tended to select against GFP expression if the brightest animals were not selected every generation. Since the Wild M5 dissecting microscope I used to select worms lacked a fluorescence light system, I found another researcher's plans for an inexpensive LED-based fluorescence illuminator for GFP. Unfortunately, inexpensive bandpass filters didn't provide a sufficient signal-to-noise ratio for viewing excretory cell processes in the 1-mm nematodes. This problem was solved by placing a Chroma bandpass excitation filter between the LED and the sample, and a pair of bandpass emission filters in the eyepieces. I also moved the filters to a standard filter cube for imaging and scoring neurons with the Axioplan 2. Additionally, although the neurons I observed did not overlap gut autofluorescence, portions of the excretory cell near the gut were difficult to assess for damage while using a longpass filter, requiring the purchase of green GFP bandpass filters which blocked the yellow gut autofluorescence and permitted clear observation of excretory cell processes. The purchase of all of these bandpass filters, funded by the Alan E. Leviton Student Research Award, solved these problems and allowed me to finish my thesis research.

My observations revealed that neurons expressing dopamine and γ-aminobutyric acid (GABA) suffered similar copper-induced damage. However, the excretory cell resisted damage by a factor of seven compared to neurons. The concentrations at which 50% of the animals had excretory cell damage (EC50) were 7.02, 4.28, and 3.74 mM CuSO4 after exposure for 10, 12, and 14 hours; EC50 values for dopaminergic neurons were 0.94, 0.67, and 0.49 mM (Figure 1).

Animals paralyzed by copper were misidentified as dead by previous researchers. Live/dead testing with the cell-impermeant nucleic acid stain, SYTOX Orange, identified dead animals more accurately. Since cations interfere with dye fluorescence, SYTOX Orange was validated in animals treated with 2–32 mM CuSO4 before heat-killing; the highest usable copper concentration was 16 mM. Subsequent live-dead testing found fewer than 5% of animals died after copper treatment up to 16 mM for 14 hours (Figure 2). Therefore, the LC50 for CuSO4 is greater than 16 mM, approximately 25 times the EC50 for toxicity in neurons. The difference in toxicity for neurons versus the excretory cell refutes the claim that copper is not neurotoxic in C. elegans because the LC50 and EC50 are too similar for copper to be specifically neurotoxic.

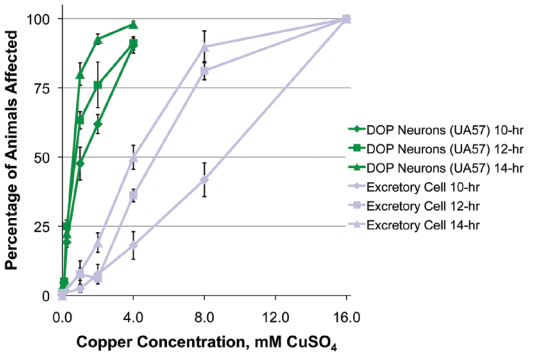


Figure 1. Effect of copper concentration and length of treatment on the survival of neurons and excretory cells.

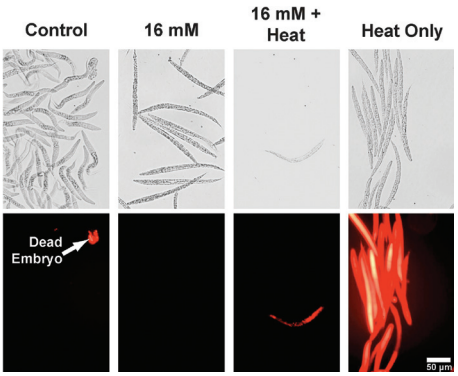


Figure 2. Sampling for live/dead animals using SYTOX Orange stain.