A Study of $NaNO_2$ Effects on *Caenorhabditis Elegans*

Mikey Rieger and Dr. Chery Whipple
Crossroads Academy, Lyme, NH
New Hampshire Academy of Science - 2017

Consumption of Sodium Nitrite ($NaNO_2$) can cause elevated blood nitrite levels in humans, induce oxidative stress and disturb metabolic pathways, particularly in infant populations. $NaNO_2$ is used in curing meat products for preservation and prevention of bacterial growth, in particular the botulinum bacterium. $NaNO_2$ occurs naturally in many vegetables with higher concentrations in leafy greens, celery and beets as well as some drinking water-sources. In this investigation *Caenorhabditis elegans* (*C. elegans*) was used as a model organism to study potential health risks of $NaNO_2$. The modulation of the activity level of *C. elegans* exposed to various $NaNO_2$ concentrations was used as a measure of their health. Using a stereomicroscope, the motion of *C. elegans* was observed and activity levels assigned on a scale between 0 and 6 (0, no movement, straight-body exhibiting death; 1-3, reduced movement; 4, normal movement, sinusoidal form; 5-6, rapid movement, roaming). *C. elegans* were exposed to $NaNO_2$ dilutions ranging from 0% to 3%, with and without *E. coli* as their food source. Five unsynchronized adult worms per dilution were analyzed with each worm treated and observed separately during the experiment. We observed the following average activity level reductions with food present: at 1 % $NaNO_2$, 70% from 4.0 to 1.2 after 4 hours; at 2% $NaNO_2$, 20% from 4.0 to 3.2 after 4 hours; and at 3% $NaNO_2$, 60% from 4.0 to 1.6 after 4 hours. The average activity level reductions without food present were: at 2% $NaNO_2$ (control group), 10% from 4.0 to 3.6 after 4 hours and at 0% $NaNO_2$ (negative control), 25% from 4.0 to 3.0 after 4 hours. The results suggest that in the presence of $NaNO_2$, *C. elegans* exhibit a difference in activity with and without a bacterial food present. Studies confirm that *C. elegans* are less likely to feed if no quality food is detected in substance and are capable of seeking out higher-quality food. At lower concentrations of $NaNO_2$ (1%) mixed with food, *C. elegans* display low activity (movement) and increased mortality (no movement). At 2% $NaNO_2$, the concentration may be high enough to be detected and food is avoided, which is displayed by near normal activity; without food, *C. elegans* display high activity levels and rapid movement (roaming) while seeking food. At higher concentrations (3%), we hypothesize that the absorption of $NaNO_2$ through the *C. elegans* cuticle (due to the small molecule size of $NaNO_2$) may play a factor in activity level reduction. Studies confirm that small-sized molecules can be absorbed through the cuticle. Our results may thus be interpreted as the combined effect of two competing mechanisms for uptake of critical quantities of $NaNO_2$. Future experiments will be necessary to exclude alternative explanations for our findings. These will involve the study of lower $NaNO_2$ concentrations, measurement of pharyngeal pumping to examine the connection between feeding levels in the presence and absence of food, as well as increased exposure time and population size.