TOXICOLOGICAL EFFECTS OF ALUMINUM AND TITANIUM NANOPARTICLE EXPOSURE IN C. ELEGANS

Casey McGuire; Dr. Kelly Salmon
New Hampshire Academy of Science
Hanover High School

Abstract

Nanoparticles, ranging in size from 1-100 nanometers, are potentially toxic to animals. Aluminum oxide nanoparticles (Al_2O_3 NPs) have been linked to brain inflammation and dopaminergic neuron loss. Titanium dioxide nanoparticles (TiO_2 NPs) can increase oxidative stress in the brain resulting in neurodegeneration. We used *C. elegans* as a model organism because their neurological system is mapped, they have an Insulin/IGF-1 Signaling (IIS) pathway that helps control inflammation, and there are relevant mutant strains. We investigated potential neurotoxic effects of Al_2O_3 NPs and inflammatory effects of Al_2O_3 and TiO_2 NPs through the IIS pathway. Our hypotheses were that both Al_2O_3 and TiO_2 NPs cause inflammation and that Al_2O_3 NP exposure would lead to dopaminergic neuron loss. Wildtype (WT) *C. elegans* were used in addition to two mutant strains for comparison. Daf-9 mutants are resistant to inflammation because of a mutation in the IIS pathway and cat-2 mutants are deficient in dopamine. An ethanol preference test was used to test the effect of Al_2O_3 NPs on dopaminergic neurons. Previous research shows that, following exposure to ethanol, WT *C. elegans* develop a preference for ethanol. Cat-2 mutants were used since they do not develop this preference to ethanol due to their dopamine deficiency. When exposed to 10 g/L Al_2O_3 NPs, WT worms had a preference index that was found to be statistically different from the control group ($P<0.05$) while the Al_2O_3-exposed *C. elegans* preference index was not statistically different than the cat-2 mutants ($p>0.05$). WT and daf-9 mutants were exposed to 1 g/L and 10 g/L of Al_2O_3 and TiO_2 NPs. The eggs laid per nematode was measured and a lipid stain was used to observe differences in inflammation. The WT and daf-9 *C. elegans* exposed to 10 g/L Al_2O_3 or TiO_2 NPs had laying rates that were statistically different from the non- exposed nematodes throughout the reproduction period ($P<0.05$). The WT and daf-9 *C. elegans* treated with 1 g/L Al_2O_3 and TiO_2 NPs had more lipids than the control treatments. The daf-9 mutants had lower lipid levels than the WT for both control and experimental groups. This study suggests Al_2O_3 NPs could have neurodegenerative effects on the dopaminergic neurons of *C. elegans*, and that Al_2O_3 and TiO_2 NPs cause inflammation in *C. elegans*. Al_2O_3 and TiO_2 NPs are used increasingly in medicine, cosmetics, manufacturing, and food industries. These particles should be tested further due to possible neurodegenerative and inflammatory effects they may have on humans.

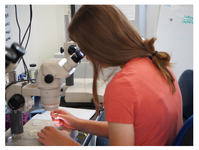
Introduction

Nanoparticles have a diameter of less than 100 nm and can cross biological barriers in the human body including the Blood-Brain Barrier.

- **Aluminum oxide nanoparticles (Al_2O_3 NPs)** are
  - Commonly used in drug delivery, material surface coatings, cosmetics, and food products.
  - Aluminum exposure linked to neurodegenerative diseases and increased inflammation in parts of the brain.
  - Aluminum exposure can lead to dopaminergic cell death/dysfunction.
- **Titanium oxide nanoparticles (TiO_2 NPs)**
  - Used in consumer goods medicines, and pharmaceuticals.
  - After exposure, TiO_2 can travel to the brain.
  - TiO_2 linked to increased production of highly reactive molecules (ROS) due to oxidative stress. ROS plays a critical role in neurodegeneration since oxidative stress.
- **Al2O3 and TiO2 NPS have been linked to neurodegeneration and increased inflammation in the body contributing to neurotoxicity.**

Caenorhabditis elegans

- 1 mm long nematodes
- Completely mapped neurological system and an Insulin/IGF-1 Signaling (IIS) pathway. Just like the human insulin pathway, the IIS pathway affects inflammation in *C. elegans*.
- **Mutant Strains used in this study**
  - Cat-2 *C. elegans* = lack an enzyme needed for the synthesis of dopamine.
  - Dopamine GFP-labeled *C. elegans* = dopamine-1 receptors labeled with green fluorescent protein.
  - Daf-9 *C. elegans* = enter dauer more often, have increased longevity and resistance to oxidative stress.

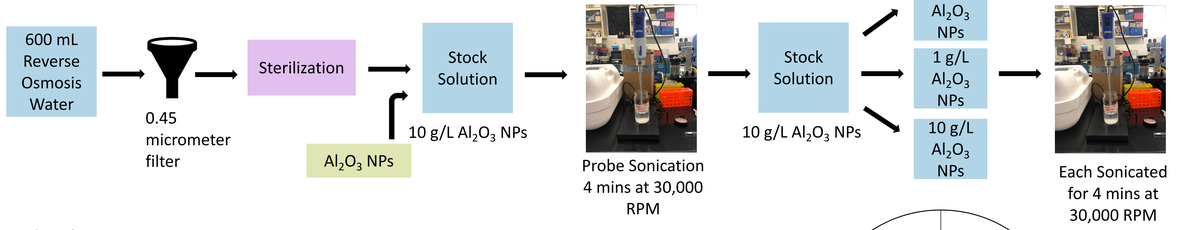


Research Aim

This study investigates the neurotoxic effects of aluminum nanoparticles along with the effect that aluminum and titanium nanoparticles could have on inflammation, specifically through the IIS pathway. The hypothesis was that both Al_2O_3 NPs and TiO_2 NPs cause increased inflammation in *C. elegans* and that Al_2O_3 NP exposure leads to dopaminergic cell death and dysfunction.

Methods and Materials

Solution Preparation



Ethanol Preference Assay

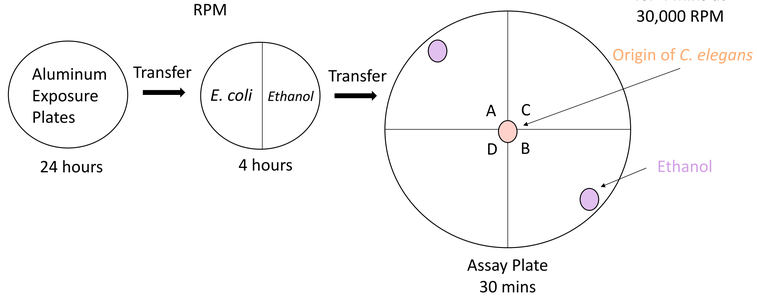
Experimental Group

- 100 Wildtype Exposed to 10 g/L Al_2O_3 NPs

Control Groups

- 100 Wildtype Exposed no NPs
- 100 Cat-2 Exposed to no NPs

Preference Index Calculated: $\frac{qA+qB-qC-qD}{total\ C.elegans\ on\ plate}$



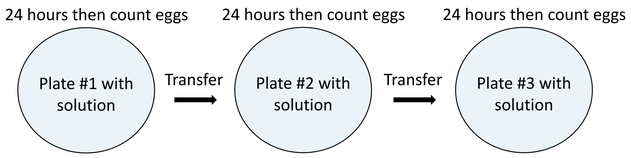
Egg Laying

Experimental Groups

- Wildtype Exposed to 10 g/L Al_2O_3 NPs
- Wildtype Exposed to 1 g/L Al_2O_3 NPs
- Wildtype Exposed to 10 g/L TiO_2 NPs
- Wildtype Exposed to 1 g/L TiO_2 NPs
- Daf-9 Exposed to 10 g/L Al_2O_3 NPs
- Daf-9 Exposed to 1 g/L Al_2O_3 NPs
- Daf-9 Exposed to 10 g/L TiO_2 NPs
- Daf-9 Exposed to 1 g/L TiO_2 NPs

Control Groups

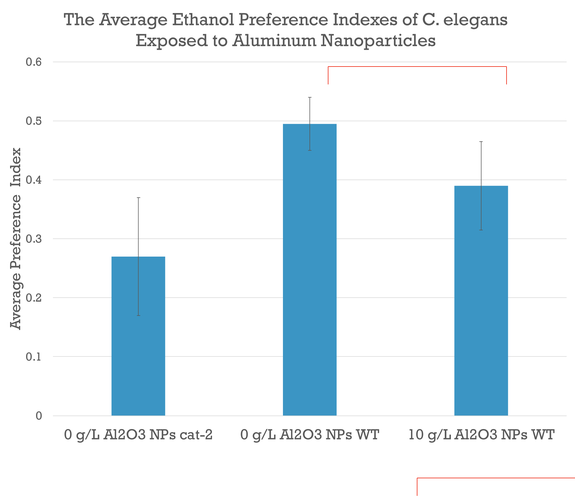
- Wildtype Exposed to to no NPs
- Daf-9 Exposed to no NPs

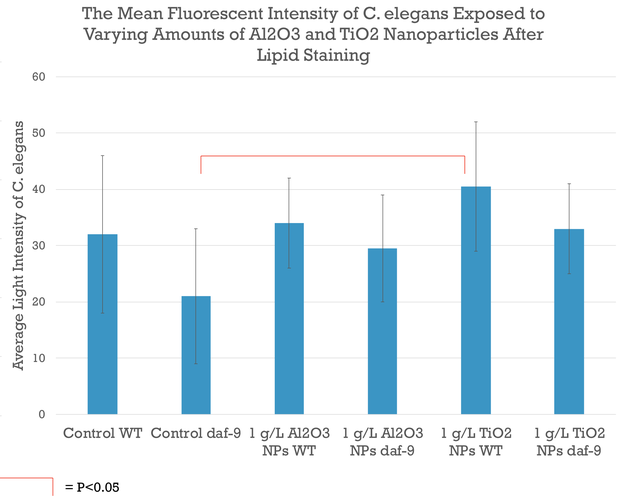


Lipid Staining

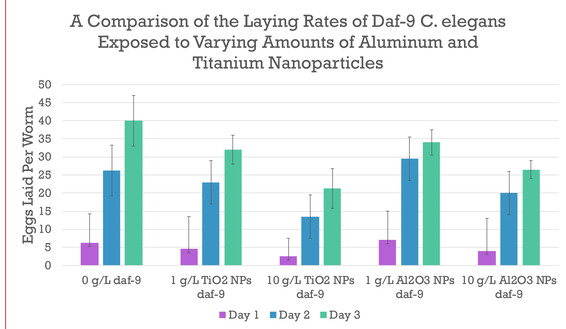
After three days of laying eggs, the *C. elegans* were exposed to RediStain™ WormDye Lipid Green. The protocol on the Nemametrix website under the product was used. Images of the *C. elegans* were taken and analyzed using Fiji - Image J software.

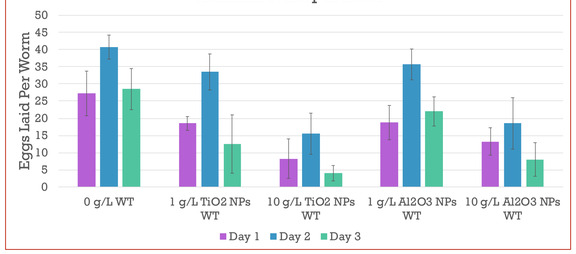
Results





Results Continued





Conclusions and Discussion

This study suggests that Aluminum could have neurodegenerative effects on *C. elegans* and that both Titanium and Aluminum NPs cause inflammation in *C. elegans.*

- The ethanol preference test suggests that Al_2O_3 NPs could cause some dopaminergic cell dysfunction in *C. elegans*.
- Increased inflammation was observed in *C. elegans* exposed to TiO_2 and Al_2O_3 NPs. The TiO_2 NPs caused slightly more inflammation than the Al_2O_3 NPs, which was seen in both the egg laying rates and lipid staining images.

Due to the prevalence of NPs in the world, people are often inhaling and consuming them. In addition, more and more applications of NPs are being uncovered making their safety a growing concern. *C. elegans* are a model organisms for humans, and thus we should be concerned if Al2O3 NPs and TiO2 NPs, some of the most commercially used NPs, could have health consequences. The potential effect of Aluminum nanoparticles on dopaminergic neurons makes research on the toxicity of these particles significant as Al2O3 NPs could be a risk factor for neurodegenerative diseases such as Parkinson's. In addition, inflammation caused by Aluminum and Titanium NPs could be contributing not only to neurodegenerative diseases, but also possibly causing an increased risk of diabetes, heart disease, and cancer.

Future Experimentation:

- Not only the laying rates of the *C. elegans* will be monitored, but in addition, the hatching rates of these eggs will be counted since hatching rates are important indicators of toxicity.
- The ethanol preference test and lipid stain will be repeated to add to the results in this study and improve their accuracy.
- The laying rate procedure will be repeated with daf-2 and daf-16 *C. elegans* in order to further explore the effect of Aluminum and Titanium nanoparticles on inflammation through the IIS pathway.

Acknowledgements

I would like to thank everyone who supported this project. Specifically, I would like to thank Kelly Salmon, my mentor, for helping with designing the process and analyzing the results. Finally, thank you to the New Hampshire Academy of Science for providing assistance and lab space to perform this experiment.