Project Proposal

Project Title: The Behavioral Effects of Microplastic Consumption in *Drosophila melanogaster*

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Project Description:

The overall aim of this project is to see how the behavior of *Drosophila melanogaster* changes in response to microplastic exposure. I plan on exposing *Drosophila* to microplastics during their egg, pupae, larva, and adult stages. After the exposure period, I will conduct assays in order to collect data on the behavior of the *Drosophila* and then compare those results to the assays I conduct on the control group.

The results of this project affect a vast majority of the human population as the exposure and consumption of microplastics increase as the world continues to produce and mismanage plastics. Even individuals who only use reusable items and biodegradable plastics will be exposed to the chemicals in microplastics as they continue to leach into the food and water that humans consume.

After exposure, I expect to see that the organisms will move around less, the organisms will eat less, and abnormal social interactions will occur. As the concentration of polyethylene microplastics increases, the change in behavior will also stray further from the control.

Background:

How does the direct exposure of polyethylene microplastics affect the behavior of *Drosophila melanogaster*? If *Drosophila melanogaster* organisms are exposed to polyethylene microplastics, then the organisms will move around less, the organism will eat less, and the social distance between the organisms will be smaller.

Plastic and Microplastic Exposure

Due to human activities, plastics are entering the oceans; eventually, they can appear in the food and water that animals, including humans, consume. There are three main types of plastic that end up in the ocean: plastic that is misplaced during movement to landfills, littered plastic that is transported by rainwater and wind, and the plastics such as microbeads and microfibers that go down the drain when cosmetics or laundry is washed (*How Does Plastic End up in the Ocean?*, n.d.). Additionally, larger pieces of plastic can eventually break down into microplastics. Once the plastics become a small enough size, many animals mistake them for food or accidentally consume them. As a result, microplastics can then travel up the food chain until they are possibly consumed by humans. The issue of microplastics is so prevalent that in 2015, it was estimated that 15 to 51 million microplastic particles were floating on surface water worldwide (Lim, 2021). Exposure to microplastics has reached the point where humans are consuming 39,000 to 52,000 particles annually (Cox et al., 2019).

However, microplastics are not the only way that plastic substances are consumed though. Individuals can also be exposed to the chemicals within microplastics as the plastics break down when exposed to heat. For example, the heat from microwaves can cause the chemicals to break down as food is reheated. Not only that, but the chemicals are broken down even more over long term use as many takeout containers are not built to be washed and reused (Zanolli, 2020).

The Effects of Microplastic Exposure

Microplastic exposure is a cause of concern as research has shown that many of the common chemicals that make up plastics have been known to have adverse side effects on humans. This list of chemicals includes, but is not limited to styrene, formaldehyde, and bisphenol a. These substances have been labeled carcinogenic to humans and have been linked to disrupting the endocrine system, reproductive system, and nervous system, among others (Husain et al., 2015).

***Drosophila* as a Model Organism**

Drosophila melanogaster, commonly known as the fruit fly, has been used for over a hundred years as a model for biomedical sciences. The low cost, short lifespan, and easy maintenance has made *Drosophila* one of the most commonly used model organisms (Tolwinski, 2017). *Drosophila* makes a good model for *Homo sapiens*, commonly referred to as humans, because the organism contains genes that have direct relation with genes that affect human disease. Out of all genes that affect human disease, more than 60% have *Drosophila* ancestor specific genes. *Drosophila* has been widely used to study brain disorders such as Parkinson's and Alzheimer's disease because the *Drosophila* exhibits complex traits similar to humans. These traits include a circadian rhythm, drug responses, locomotion, aggressive behavior, and longevity (Mackay & Anholt, 2006). In addition, *Drosophila* animal welfare ethical review boards do not have to approve research involving the organism (Baenas & Wagner, 2019).

Polyethylene Wax Particles

Polyethylene was chosen as the plastic type for this project because it is the most widely used plastic in the world (*Polyethylene | Properties, Structures, Uses, & Facts | Britannica*, n.d.). Additionally, most plastic food containers, both takeout and reusable containers, are made out of either low-density polyethylene or polypropylene (Zanolli, 2020). Polyethylene was chosen over polypropylene because it is one of the most widely used polymers to produce plastic (Horton et al., 2017).

For this project specifically, polyethylene wax (PE wax) particles will be used. The PE wax particles are 8-10 microns long and thus will be small enough for the *Drosophilae*, normally 3 mm long and 2 mm wide, to possibly eat (Miller, 2000). At the very least, the *Drosophilae* will be exposed to the microplastic for the majority of its lifespan.

PE wax is a thermoplastic polymer consisting of long ethylene monomer chains. PE wax can come in both high-density polyethylene and low-density polyethylene (*POLYETHYLENE WAX*, n.d.) The material has applications such as a plastic additive, lubricants, resin additive and more. The singular component of PE wax, ethylene ($H_2C{=}CH_2$), is the simplest of the alkenes, organic compounds that contain carbon-carbon double bonds. Sources of ethylene include natural gas and petroleum as well as a naturally occurring hormone in plants. The polymerization of ethylene monomers results in the plastic polyethylene (*Ethylene | Structure, Sources, Production, Uses, & Facts | Britannica*, n.d.).

Significance

Much of the established research on the impacts of microplastic have focused on the behavioral and physical changes that occur. Even among similar organisms, exposure to plastic can result in varying results. For example, some studies have found that feeding behavior in amphipods, copepods, and coral species is impacted by microplastic exposure. In contrast, no effect on feeding behavior was found in shore crabs (Cunningham et al., 2021). Copepods and shore crabs are both crustaceans, yet the two organisms yielded different results. Additionally, it is very likely that a greater concentration of microplastics would result in a more severe change (Personal Communication, E. Cunningham, 2022).

This project chooses to focus on the behavioral effects of microplastic exposure as physical changes in humans related to cancer-causing agents have been widely studied, but less information is known about the behavioral changes (Husain et al., 2015). Changes in behavior could have significant implications as it could result in susceptibility to various mental health illnesses. This project builds upon previous microplastic behavior research and connects it to human behaviors.

Research Plan:

Materials List:

- *Drosophila melanogaster*
- Materials to cultivate and care for the *Drosophila*
    - Food– dehydrated media from Carolina Biological
    - Vials
    - Anesthesia– FlyNap from Carolina Biological
    - Water
    - Yeast
- Polyethylene wax particles
- Camera
- Capillary tubes
- Sugar water
- Food dye
- Petri dishes
- Graduated cylinder

Independent Variable:

- The microplastic concentration the *Drosophilae* is exposed to

Dependent Variable:

- The behavior of the *Drosophilae*

Standardized Variables:

- Light exposure/circadian rhythm of the *Drosophilae* 12 hr light/12 hr dark
- Media that the *Drosophilae* are consuming
- Air pressure
- Temperature
- Exposure time to the microplastics

Methods:

Culturing *Drosophila*:

Adult *Drosophila* from a culture vial will be anesthetized and separated by gender and placed with a ratio of three females to one male in a new culture vial with hydrated media that will or will not contain the independent variable (*An Introduction to Fruit Flies*, 2015).

Preparation of microplastics:

The plastic will be acquired with the particles already at the correct size. The concentrations the *Drosophila* will be exposed to will correspond with the no observed adverse effect level (NOAEL) of 50 μg/mL recorded by Sun et al. (2021). Then, two larger concentrations will be created based on that concentration. The microplastics will be mixed into the water used to hydrate the media the *Drosophilae* consume.

Locomotion assay:

The assay used to quantify motion will be based on the assay created by Madabattula et al. (2015). 20 *Drosophilae* will be placed into a graduated cylinder with a line marked 17.5 cm up the tube. The *Drosophilae* will be tapped to the bottom and once the last tap occurs, a 2 minute trial will be conducted and recorded. Afterwards, the video will be analyzed to record the number of *Drosophilae* that climb above the 17.5 centimeter line every 10 seconds. After each trial, the *Drosophilae* will be replaced; 5 such trials will be conducted. This assay makes use of the negative geotaxis, the natural behavior of *Drosophilae* to move in the direction opposite of gravity (Madabattula et al., 2015).

Feeding Assay:

A capillary feeding assay modeled after the CApillary FEeder assay (CAFE assay) created by Diegelmann et al. (2017) will be used for this project. For the assay, four capillary tubes filled with dyed sugar water will be placed into the fly vials containing the *Drosophilae*; then, after a feeding period, the amount of sugar water remaining in the capillary tube will be recorded.

Social Space Assay:

In order to quantify social behavior, an assay based upon the social space assay created by Simon et al. (2012) will be used; social space is the distance between two individuals of the same species. Social space is determined by the ideal balance of attraction and repulsion (Kaur et al., 2015). The majority of Canton-s flies, regardless of gender, lie within two body lengths from each other (Simon et al., 2012)

The *Drosophilae* will be allowed to grow up together with interactions with both genders in order to be socially enriched. The day prior to experimentation, the *Drosophilae* will be separated by gender. The following day, 30 *Drosophilae* of the same gender will be placed into a petri dish measuring 9 cm in diameter and 1.4 cm in depth. The *Drosophilae* will be given 15 minutes to acclimate to the testing chamber then after 15 minutes, a photo will be taken and digitally analyzed to measure the distance between a *Drosophila* and its nearest neighbor.

General Assay Information:

The assays for the control group will be conducted first in order to gather preliminary data and practice handling the model organism. These three assays were chosen because they model behaviors seen in neurodevelopmental disorders such as autism and in various mental illnesses such as depression and anxiety.

Risk/Safety Concerns:

Currently, there are no safety concerns. The culturing of the model organism and conducting the assays will be done under the supervision of my advisor, Dr. Crowthers, in the Mass Academy labs; the labs at Mass Academy have a biosafety level of two.

The microplastic concerns are addressed in the separately attached safety data sheet.

Data Analysis:

In order to verify the results of the experiments, a t-test will be conducted to prove the significance of the data. The correlation between polyethylene concentration and the assay parameters will be conducted through a linear regression analysis.

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<u>Timeline:</u>