The Behavioral Effects of Microplastic Exposure in *Drosophila melanogaster*

In the status quo, it has been estimated that there are 15 million to 51 million microplastic particles floating on surface water worldwide (Lim, 2021). The use and production of plastics have increased greaty that by 2019, humans were producing 460 million tons annually (Ritchie & Roser, 2018). These plastics arrive in the ocean in three main ways: plastic that is mishandled during movement to landfills, littered plastic that is transported by runoff and wind, and plastics such as microbeads and microfibers that go down the drain when things such as cosmetics and or laundry are washed (*How Does Plastic End up in the Ocean?*, n.d.). In addition, larger pieces of littered plastic can be broken down into microplastics. These plastics can become small enough for an organism to eat as they mistake the plastic for food. Once an organism consumes the plastic, it can travel up the food chain all the way up to human consumption. Exposure to microplastics has increased to the point where humans are consuming 39,000 to 52,000 particles annually (Cox et al., 2019).

But microplastics are not the only way that substances in plastic are consumed; when plastics are exposed to heat—in a microwave for example—the chemicals in microplastics can break down and enter the food humans consume. On top of that, the chemicals can be broken down even further over long-term use as many takeout containers are not built to be washed and reused (Zanolli, 2020)

The Effects of Microplastic Exposure on Humans

The increased exposure to microplastics is alarming as research has shown that many of the common chemicals that make up plastics have been found 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, nervous system, and more (Husain et al., 2015).

Previous Research and Knowledge Gap

Most of the prior research into microplastic exposure has either investigated the physical effects in humans or the behavioral effects in model organisms. The research involving model organisms has found that even among similar species, exposure to microplastics can have varying results. For example, some studies have found that feeding behavior in amphipods, copepods, and coral species decreases after 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, the effects found could likely be even more severe if the organisms were exposed to a higher concentration of microplastics (Personal Communication, E. Cunningham, 2022).

This project chooses to focus on the behavioral effects of microplastic exposure rather than physical effects as physical effects in humans have been widely studied (Husain et al., 2015). In addition, behavioral changes in humans could have significant implications as they could lead to susceptibility to various mental health illnesses. In order to link a change in behavior due to microplastic exposure, *Drosophila melanogaster*, will be used as a model organism.

***Drosophila* melanogaster as a Model Organism**

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

Polyethylene Wax Particles

Polyethylene was used as the microplastic for this project as polyethylene 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 ultimately chosen over polypropylene because it is used more widely than polypropylene (Horton et al., 2017).

Specifically, polyethylene wax (PE wax) particles were used in this project. The PE wax particles utilized were 8-10 microns long and thus were small enough for the *Drosophila*—on average 3 mm long and 2 mm wide—to possibly consume (Miller, 2000). Even if the *Drosophila* did not end up consuming the particles, the organisms were exposed to the microplastics for the majority of its lifespan.

PE wax is a thermoplastic polymer consisting of long ethylene monomer chains. PE wax can be made with both high-density polyethylene and low-density polyethylene (*POLYETHYLENE WAX*, n.d.) The material has applications such as a plastic additive, lubricant, 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. Polyethylene plastic is the result of the polymerization of ethylene monomers (*Ethylene | Structure, Sources, Production, Uses, & Facts | Britannica*, n.d.).

Researchable Question

How does the direct exposure to polyethylene wax microplastics affect the behavior of *Drosophila melanogaster*?

Objective

In order to address the researchable question, three assays—feeding, locomotion, and social behavior—were conducted with *Drosophila* that had been exposed to the PE wax particles, and *Drosophila* that had not. The microplastic exposure was conducted with three different exposures and ranged from the moment the *Drosophila* eggs were born to the adult stage. After the assays were completed, a t-test was conducted in order to validate the significance of the data. Additionally, a linear regression test was conducted in order to quantify the correlation between polyethylene concentration and the assay results.

The three assays were chosen for this project because they gather data on three vital behaviors for human well-being and survival: locomotion, feeding, and social interactions (Glover, 2017; Petrovich, 2018; Young, 2008).