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Article

Effects of common pesticides on population size, motor function, and learning capabilities in *Drosophilia melanogaster*

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SUMMARY

In this study, we aim to examine the effects of commonly used pesticides on population size, motor function, and learning in *Drosophila melanogaster*. Specifically, we examined the effects of metolachlor, glyphosate, chlorpyrifos, and atrazine on *Drosophila*. Pesticides are toxins used to control pests and weeds in crops and in the past, have been connected to multiple health issues in those exposed. Overall, the results were collected using a negative geotaxis assay, aversive phototaxis assay, and a larval learning assay whose data was averaged. This project can be applied to the 1.8 billion people who are exposed to pesticides and assist in defining the connections in between pesticides and the tested diseases.

INTRODUCTION

Pesticides are products intended to kill or repel unwanted plants or animals. They can be categorized by their intended target; herbicides (weed killers), insecticides (bug killers), and fungicides (fungus killers) are all types of pesticides. While they are commonly used in many settings around the world, farmers rely particularly heavily on pesticides to protect their crops from weeds and insects. However, a consequence of using pesticides is the fact that some of it is washed off the farm to surface water. This contaminated water may be toxic to animals that live in it, as well as to humans. Since the 1940s, farmers have been forced to increase the strength of pesticide treatments in order to effectively kill their targets. This occurs because of "pesticide treadmill," a phenomenon in which plants or insects evolve immunity to a particular pesticide treatment conditions and farmers must employ more and more extreme measures to overcome this resistance. Ultimately, this results in higher concentrations of pesticides in surface water and more exposure by humans and other animals.

Pesticide exposure has been linked to cancer, reproductive harm, and health issues in children (2). Other recent studies have concluded that organophosphorus and organochlorine pesticides are linked to a variety of human diseases and disorders, including ADHD, Alzheimer's disease, Parkinson's disease, and birth defects (5). Neurodegenerative diseases affect the brain; many are fatal and have no cure. These illnesses complicate daily tasks, using medicine or surgery can help slow the progression, eventually causing severe disability (7). About one in six children in the U.S. has a developmental disorder, some more than one. Scientists hypothesize that toxic chemicals in the environment can be taken in by the mother and may cause the child to have developmental or neurological issues ranging from learning problems to intense behavioral or emotional disorders. (6). Birth defects are physical derangements that occur before a baby is born. The cause of these diseases have been credited to infections, genetics, and certain environmental factors, like pesticides. Connections between these disease and pesticides have presented themselves in the past with people who would have constant exposure to pesticides. The best example that encompasses the three conditions being tested took place in 2005. Three women and their husbands lived and worked at a large farm that would have allowed constant exposure to pesticides. The women each had kids, and all of them developed some sort of health deficits within 5 years of birth. As they grew older, the adults all began to experience symptoms of neurodegenerative diseases, and it lead scientists to connect it back to their work life at the farm. Since then, research over pesticides has been much more concentrated on its affect towards humans then before.

This study aims to examine the short-term and long-term effects of pesticides on population size, motor function, and learning capabilities in *Drosophila melanogaster. Drosophila* are a species of fruit flies commonly used for scientific research. The genome of this species is 75% identical to the genome of humans, making *Drosophila* an effective model system for biological studies. In addition, this species is a viable model organism for transgenerational experiments, as a single pair of flies can produce hundreds of offspring in a short period of time and both females and males become sexually mature within a week of becoming adults or ten days of life.

The pesticides used within the parameters of this project are atrazine, metolachlor, glyphosate, and chlorpyrifos. Atrazine and glyphosate are herbicides used primarily to manage corn crops, yet the risks induced by these chemicals caused them to be banned from use in certain parts of the world. Both of these, as well as the insecticide chlorpyrifos, are based off of phosphorous compounds, making them organic phosphorous compounds (organophosphates). Metolachlor, another common herbicides, is an organochlorine (based off of a chlorine compound). Each pesticide has been shown to cause a broad range of adverse effects. Short-term exposure is generally associated with nausea and vomiting, while long-term impacts may vary by pesticide. Organophosphates have been known to cause memory problems, while organochlorines target motor functions. We hypothesize that exposure to atrazine, metolachlor, glyphosate, and chlorpyrifos will have a negative effect on viability, motor function, and learning capabilities in *Drosophila melanogaster*. In this study, we will assess the effects of these pesticides on viability by measuring population size, motor function using the negative geotaxis assay, and learning with the aversive phototaxis suppression assay and the larval learning assay. The *Drosophila* will be tested and given the pesticides through means of ingestion and the control group will be the flies given normal food without any pesticides.

RESULTS

The *Drosophila* were fed their designated pesticide food for 14 days before the data was collected. The treatment groups were chlorpyrifos, atrazine, metolachlor, glyphosate, and the control group that was left untouched by pesticides. Ten *Drosophila* were assigned to each trial and ten trials of each assay and pesticides were conducted. Population size was monitored by counting the amount of *Drosophila* each day using carbon dioxide to anesthetize them.

Population size

Population size was monitored by counting the amount of adult *Drosophila* everyday during the 14-day span of testing. The population of *Drosophila* greatly decreased in both the metolachlor and glyphosate settings. On the final day, the amount of *Drosophila* remaining was compared to the starting population on the first day of testing, imposing the inference that these two pesticides had a greater effect towards the longevity of the *Drosophila*.

Motor function

We performed the negative geotaxis assay to measure the effect of pesticides on motor function in *Drosophila*. This test relies on the flies' natural geotaxis instincts, which is to move upward without need for stimulation. After 10 seconds, the amount of flies passed in the 8 cm line on the vial were counted to produce the results for each pesticide for decreased motor function. In the negative geotaxis assay, we found that flies treated with all of the tested pesticides demonstrated impaired motor function compared to untreated flies (see **Figure 1**). The flies that were exposed to metolachlor significantly showed less motor functions than the ones introduced atrazine. Unexpectedly, the flies exposed to the pesticides that were not previously researched had a lesser capability to climb the designated height, allowing the possibility to conclude that metolachlor, being the most unstudied and the one having the most effect, had a greater impact towards the flies' motor functions.

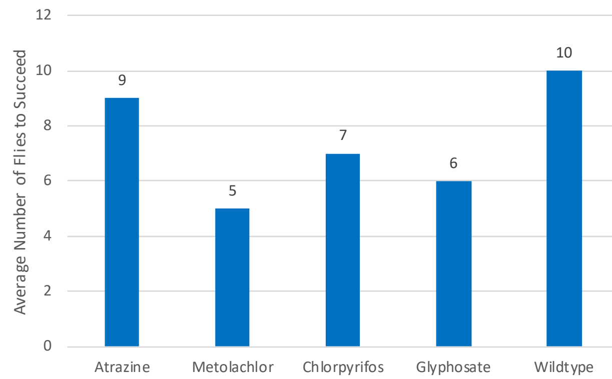

Figure 1: Effect of pesticide exposure on motor function. The negative geotaxis assay tested the motor function of adult *Drosophila*, measuring the effect on the neural synapse bridge by measuring motor function speed. This figure depicts metolachlor having a slower response time to the control variable, indicating a specific effect towards the nervous system of the *Drosophila*.

Learning

The most important assay was the aversive phototaxis suppression assay, which tested whether the adult flies ability to retain information shortly learned before. This test watched for the flies not being able to hold information from the sugar reward test and using that information for a stimulant test. The results showed that their incapacity to retain information that was learned a short while before seemed to be very prevalent within the metolachlor variable assays described in **Figure 2** (p-value < .00001, calculated by a t-test). Without a doubt, the metolachlor had the heaviest impact on the adult *Drosophila*'s capability to remember information compared to the control.

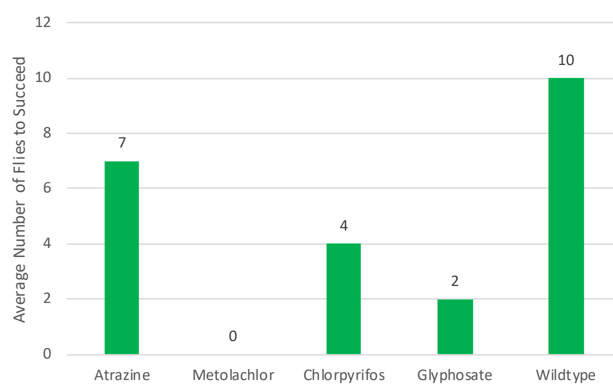

Figure 2: Effect of pesticide exposure on ability to retain information. Age-synchronized *Drosophila* adults were put in a 3D T-maze (n = 10) and were trained using a rewards system to gear away from their normal stimuli. After the time passed, the number of flies in the correct stimuli were counted and the ones that "failed" were averaged. *Drosophila* exposed showed a significant drop into the successful results of chlorpyrifos and metolachlor (p-value < .00001.)

Transgenerational effect

Lastly, the larval learning assay using a baiting technique in order to test flies in their larvae stage. This test measured for the larvae's capacity to follow olfactory stimulants that would normally be easily distinguished. After testing, it was found that the ones exposed to both metolachlor and chlorpyrifos both were greatly unable to follow the simple stimuli and go to the sugar reward (**Figure 3**), so it can be concluded that the metolachlor, which had a greater impact, showed the most harm towards the flies exposed (non-significant results for a *p* > 0.05). These results could suggest that atrazine caused the least effect towards the proceeding generations.

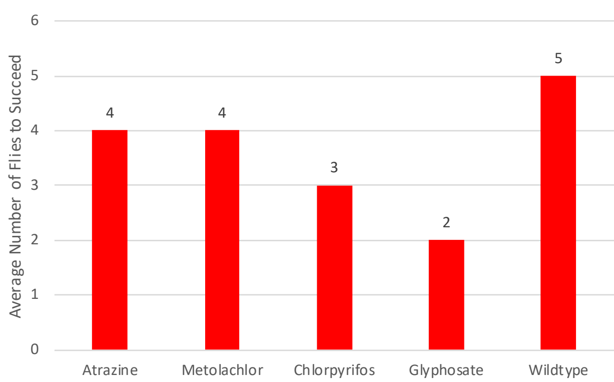

Figure 3: Effect of pesticide exposure on larval learning assay performance. Age-synchronized *Drosophila* larvae (n = 10) were placed on a plate and allowed to move towards aversive and positive olfactory stimuli. At the end of the assay, the number of flies that had moved towards each stimulus was counted, with flies that had moved toward the positive stimulus considered "successful." Flies exposed to metolachlor and chloropyrifos showed decreased assay performance, although this was not statistically significant ($p > 0.05$).

DISCUSSION

This project came to very unexpected results. Instead of being the most harmful as expected, the atrazine had the least impact compared to all the other pesticides while metolachlor had the most. The reason behind this could have been attributed to multiple factors, but the main one would have been the most crucial: while atrazine is the most deadly known, there are many other pesticides that haven't been studied yet because of the amount in pesticides. But the biggest factor could be attributed that the toxicity of the other pesticides, such as metolachlor and glyphosate, in smaller amount has a greater toxicity that the large amount of the most common ones. Another potential factor could be the higher concentration of chlorine in the base of metolachlor that atrazine, glyphosate, chlorpyrifos lack, strengthening its toxicity. Most organophosphates ended up having a strong affect towards the memory assay of the aversive, yet in most assays, metolachlor surpassed the other variables. Lastly, glyphosate had an aversive effect towards the *Drosophila* out of any of the other organophates tested, another reason for the strength could have been the fact that it is based off of glycine instead of esther, giving it a stronger based.

We encountered several limitations during experimentation. Although the flies were maintained in a controlled environment, their consumption of pesticide-treated medium could not be controlled. Another limitation in this study is that the anesthesia used on the flies could have had an effect on motor function and learning capabilities of the flies in addition to the effect of the pesticide treatment. The potential effects of anesthesia on flies' movement and learning were controlled as much as possible by allowing a minimum five minute acclimation period following exposure to anesthetic. Additionally, by comparing the treated flies to the untreated, it highlights the negative factors of the pesticides in any aspect, because the toxin will most definitely have an effect. In addition, carbon dioxide (CO_2) was used as the anesthetic in these experiments instead of Flynap TM, because CO_2 has a shorter anesthetic period. Another limitation in these experiments is the potential toxicity of the pesticides; for example, if the pesticide treatments are severely toxic to *Drosophila*, the doses used may have impacted their ability to perform on the assays due to reasons other than impairment of motor function or learning capabilities. However, we accounted for this limitation by calculating an appropriate dosage for *Drosophila*. Finally, designing and 3D-printing the prototype for the aversive phototaxis suppression took longer than expected,

Future research could be conducted to show the effects of other common pesticides, or to further investigate the specific neural pathway that the toxins affect. Some toxins, such as organophosphates and organochlorines, are already known to target specific synapses and destroy connections between transmission neurons. Also, it could be researched whether there is a different effect between genders, and the specific way that it affects both. Different medium types and model organisms could also be tested, along with time of exposure and mode of exposure (not just by consumption).

New studies have shown that 1.8 billion people globally per year are exposed to pesticide, both in household products for gardening or weed killers used on farms. Individuals who live in areas surrounding farms or agriculture may inhale aerosolized pesticides, and farmers may come into contact with pesticides when harvesting crops or pulling out weeds. This is also a concern for the next generation, as individuals who have come into with toxins through skin absorption or inhalation, may carry the poison in their system, and when they have children, they may pass on the built-up toxins, which can affect the child's health. In general, the health and methods of th eagricultural system could be completely revolutionized with continuation of such research in efforts to better the way that farms are run along with the population's health.

MATERIALS AND METHODS

Safety

First, these pesticide toxin extracts may be harmful to humans if the liquid form comes to contact with the skin or is inhaled. Multiple measures were taken for security including goggles, gloves, lab coat, and a filter face mask. The pesticide powders were handled under the fume hood to further provide protections. At the end of the experiment, the diluted pesticides were disposed of in the South Household Hazardous Waste Drop-off.

Animals

The flies were kept at room temperature in a monitored 12 hour light/12 hour dark cycle. Transfer between vials with new medium was done every three to four days to assure no risk of bacterial infiltration of the food. They were all supplied by Carolina Biological and were listed under the wildtype strain named Oregon-R. Before they were trialed, they lived in the vials with different media for about 14 days in a correspondent light/dark cycle, each group using about 10 flies in the vial. *Drosophila* were fed with 15 mL of distilled water and blue medium from Carolina Biological in shatterproof vials.

Experimental setup

The experiment was set up over a 15-day period to test short-term exposure. On the first day, the medium powder was separated into five different vials that would be changed throughout the testing period every three days. Aside from the medium, the distilled water poured into each had the designated pesticides incorporated: atrazine, metolachlor, glyphosate,and chlorpyrifos (Sigma Aldrich). Each toxin was diluted in water through serial dilutions to 0.0002 micrograms/milliliter from 100% (1 g of toxin powder in 1 mL of dH_2O) concentration to represent the dilution of what a normal human would be exposed to per square acre of land.

Aversive phototaxis suppression assay

After transferring the flies from the medium vials into the aluminum-covered side of the 3D printed T-maze (designed by the researcher) by using carbon dioxide anesthesia, flies were allowed to wake up and acclimate for five minutes. The movable trapdoor in the middle was left closed so they were unable to pass to the light side. To train the *Drosophila*, the flies were left in the dark side which was covered by aluminum and a gooseneck lamp was attached to the opposing side (which the flies could not enter) with no olfactory stimuli. The flies were counted based on how many made it to the light side once the trap door opened, reflecting their ability to detect visible stimuli of the light, which they should normally be attracted to. The ones that moved towards the light stimulation are viable for further testing and can be transferred back into the dark side. This will occur until there are ten testable flies in the dark side all coming from the same variable setting. The rest of the flies were returned back to the medium vial for longevity and transgenerational studies. For the flies that are in the T-maze, filter paper with 20 uL of aversive stimuli (quinine hydrochloride) was added to the light side. The lamp was turned on again with the open trapdoor. After one minute, the number of flies that have diverted away from the light side because of the aversive stimuli were counted as passes and the flies that remained there were counted as fails. Retest 10 different flies from each variable each trial.

Negative geotaxis assay

Ten flies were separated into five different vials with no medium and were labeled according to the pesticide treatment: metolachlor, glyphosate, atrazine, chlorpyrifos, and non-treated. After being transferred using carbon dioxide to a 50 mL conical tube, the 8 cm line was marked (calculated based off of standard height per second) and then the flies were allowed to acclimate for five minutes in the tubes. After the five minutes passed, the flies were woken up using sugar bait. The vials were tapped lightly on the counter to let all the flies fall to the bottom. A timer was set for ten seconds and the number of flies that surpassed the 8 cm mark line were counted.

Larval learning assay

The flies to sleep in each variable vial using carbon dioxide and 10 age-synchronized larvae were collected and transferred to a half fructose agar / half agarose plate with set stimuli. The aversive stimuli, concentrated octanol (Sigma Aldrich), was placed in Teflon containers with pre-poked fragrance releasers. One octanol container was located in the agarose side of the plate. On the opposing fructose agar side, a positive stimuli of amyl acetate (made with paraffin oil) was placed in the Teflon containers. After insertion of the containers, larvae were placed in the middle of the plate. Once the timer was started, the plates with larvae were left in a lighted area for five minutes. Then, the number of larvae on each side of the plate was counted.

Analysis

The results of the assays were analyzed for mortality and decreased function in comparison to the non-treated or non-altered flies. The information was input into an Excel sheet and the total mortality rate was calculated based on number of deceased flies and total fly population along with each assay's individual results.

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REFERENCES

1. Miller, Conrad. "*Drosophila Melanogaster.*" *Animal Diversity Web*, animaldiversity.org/accounts/Drosophila_melanogaster/.
2. "Pesticides 101." *MSD Manual*, www.panna.org/pesticides-big-picture/pesticides-101.
3. Powell-Hamilton, Nina N. "Overview of Birth Defects." *MSD Manual*, www.merckmanuals.com/home/children-s-health-issues/overview-of-birthdefects/overview-of-birth-defects.
4. Ganzel, Bill. "How Insecticides Work." *Living History Farm*, The Ganzel Group, 2009, livinghistoryfarm.org/farminginthe70s/pests_06.html.
5. "Attention-deficit Hyperactivity Disorder (ADD,

ADHD).” *MSD Manual*, www.msdmanuals.com/home/SearchResults?query=Attention-Deficit%2fHyperactivity+Disorder+(ADD%2c+ADHD)&icd9=314.0%3bMM667.
6. Sulkes, Stephen Brian. “Learning Disorders.” *MSD Manual*, www.msdmanuals.com/home/children-s-health-issues/learning-and-developmentaldisorders/learning-disorders.
7. Huang, Juebin. “Alzheimer’s Disease.” *MSD Manual*, www.merckmanuals.com/home/brain,-spinal-cord,-and-nerve-disorders/deliriumand-dementia/alzheimer-disease
8. “What Is Farm Runoff Doing to the Water? Scientists Wade in.” Produced by Abbie Fentress Swanson, 5 July 2013. www.npr.org/sections/thesalt/2013/07/09/199095108/Whats-In-The-Water-Searching-Midwest-Streams-For-Crop-Runoff. Transcript
9. “ISEF Rules Wizard.” *Society For Science and The Public*, ruleswizard.societyforscience.org.