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Responses to Fine Particulate Matter (PM2.5) Exposures in two Wild-Type Zebrafish Strains

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RESPONSES TO FINE PARTICULATE MATTER ($PM_{2.5}$) EXPOSURES IN TWO WILD-TYPE ZEBRAFISH STRAINS

By

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Finally, I want to thank Colton and Collin Necaise for their support throughout the drafting of this thesis. They didn't do anything, but I still appreciate it.

ABSTRACT

Responses to Fine Particulate Matter ($PM_{2.5}$) Exposures in Two Wild-Type Zebrafish Strains

Atmospheric fine particulate matter ($PM_{2.5}$) exposure poses great health risks across the globe, causing both acute and chronic illnesses in humans. Therefore, a more complete understanding of the mechanisms in which $PM_{2.5}$ induces these adverse health effects is urgently needed. Oxidative stress due to PAHs and other common components of $PM_{2.5}$ is a proposed mechanism for its adverse health effects. However, little is known about the actual mechanisms of $PM_{2.5}$ damage in humans. This study aimed to distinguish behavioral differences in two lines of zebrafish (AB & 5D) as a result of developmental exposure to $PM_{2.5}$ in order to better understand variations in its effects across these two different genetic lines. For consistent testing of a chemically similar mixture of $PM_{2.5}$ a standard reference material (SRM2786) in dimethyl sulfoxide (DMSO) was prepared for the whole particle suspensions (WPS) and soluble fractions. Embryos were exposed to varying concentrations of SRM (0, 12.5, 25, 50, 100, & 200 μg/mL). After 5 days post-fertilization (dpf), the total movement of each treatment and control group was compared across 3 light phases, each lasting 5 minutes. Our results showed that the fish of the 5D line contained more instances of significantly different behavior ($p \leq 0.05$), as well as showed more consistent and sensitive responses to $PM_{2.5}$ exposure. Also, embryos raised in the WPS solution had more instances of significant differences from the control group than those raised in the soluble fraction. Also, most of the significant differences occurred during the Dark Phase with a few occurring during the second light phase and none observed during first light phase. Our results show that the two strains react differently to $PM_{2.5}$ and that the DMSO soluble chemicals in $PM_{2.5}$ are important to in altering behavior as well as large, insoluble particles.

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ABBREVIATIONS

DMSO	Dimethyl Sulfoxide
DPF	Days Post-Fertilization
HPF	Hours Post-Fertilization
PAH	Polycyclic Aromatic Hydrocarbon
$PM_{2.5}$	Fine Particulate Matter
ROS	Radical Oxygen Species
SCH-23390	D_1 Receptor Antagonist
SiNPs	Silica Nanoparticles
SRM	Standard Reference Material 2786
WPS	Whole Particle Suspension

INTRODUCTION

1.1 Criteria air pollutants

Air pollution can be defined as any substance introduced into the environmental air that contributes to adverse health effects in humans and other organisms. According to the EPA, there are six common types of air pollutants, known as "criteria" air pollutants[1]. Criteria air pollutants, or simply "criteria pollutants," fall into the six categories of ground-level ozone, particulate matter, carbon monoxide, lead, sulfur dioxide, and nitrogen dioxide. This thesis focuses specifically on particulate matter (PM), a mixture of solid and liquid particles that can be found in the air. These particles are too small to be seen with the naked eye and can only be detected through an electron microscope.

1.2 Fine particulate matter

Fine particulate matter ($PM_{2.5}$) is a common factor in air pollution known to cause adverse health effects in many different species and is becoming a larger concern to global health, especially in urban areas. However, the process by which $PM_{2.5}$ causes these effects is largely unknown. The term $PM_{2.5}$ refers specifically to particulate matter measuring 2.5 microns or less in aerodynamic diameter and can either be naturally occurring or human-made[1]. Human-made $PM_{2.5}$ is often expelled into the environment through the combustion of solid and liquid

fuels that power industrial and domestic machinery such as engines or heating elements while naturally occurring $PM_{2.5}$ results from events such as wildfires[1].

1.3 Common $PM_{2.5}$ Constituents

The chemical composition of $PM_{2.5}$ is highly variable as this classification is typically made by particle size only. $PM_{2.5}$ includes particles such as hydrocarbons, those with carbon cores with attached metals, and secondary particles formed from oxides of sulfur and nitrogen[2]. These groups of particles can lead to numerous health complications on human development through their entrance into the bloodstream through the alveolar tissue, leading to systemic inflammation, myocardial dysfunction, and alveolar damage, among others[3]. Understanding more about the constituents of $PM_{2.5}$ and their associations with human health is a growing area of interest.

$PM_{2.5}$ can be separated into DMSO soluble and insoluble fractions by extraction after centrifugation of a sample. DMSO is a commonly used solvent for collecting organic compounds. Common chemical constituents found in the DMSO soluble fraction include PAHs, alkanes, alkanols, and other hydrocarbons. Insoluble fractions often contain more dense particles visible to the naked eye, potentially having differing effects than those of the soluble fraction on developing zebrafish by exposure.

1.4 $PM_{2.5}$ Adverse health effects

Although $PM_{2.5}$ health effects are not very well understood, oxidative stress is a proposed mechanism for the observed health effects following $PM_{2.5}$ exposure. Oxidative stress is known to be caused by components of $PM_{2.5}$ including polycyclic aromatic hydrocarbons (PAHs), a large group of ubiquitous known environmental pollutants, and metals[4]. Oxidative stress can be harmful to organisms due to an imbalance between antioxidants and radical oxygen species (ROS), allowing for the ROS to react easily with other molecules, causing more instances of potentially harmful oxidation.

The health effects of $PM_{2.5}$ exposure are wide ranging as well due to the many bodily processes damaged by continuous exposure. Specific effects of this environmental exposure include higher rates of cancer, diabetes, neurodegenerative diseases, autism, and heart disease, among others[5]. In human epidemiological studies, ambient PM has been strongly associated with many cardiovascular, pulmonary, and neurological processes, among others. Cardiovascular problems correlated with $PM_{2.5}$ exposure in humans often include vascular disfunction, hypertension, ischemic stroke and more. $PM_{2.5}$-caused cardiovascular disease occurs through direct toxicity (exposure in the bloodstream) and indirect mechanisms (induced systemic inflammation and oxidative stress)[6]. Neurological problems correlated with $PM_{2.5}$ exposure include Parkinson's disease, Alzheimer's disease, and autism spectrum disorder[7]. Finally, $PM_{2.5}$ has been strongly correlated with disrupting pulmonary function, leading to increased likelihood of lung cancer, respiratory diseases, asthma, chronic obstructive pulmonary disorder, and more[8].

Adverse health effects of $PM_{2.5}$ exposure particularly during development are also of concern for multiple reasons. During the early stages of life (fetus to early childhood), studies show various other effects of exposure specific to this time period. For example, some studies show positive correlations between $PM_{2.5}$ exposure and preterm birth[9]. Maternal $PM_{2.5}$ exposure

in mice was found to induce impaired cerebral cortex development in offspring[10]. Others found similar correlations for low birth weight and general negative birth outcomes[11,12]. Further, human studies show behavioral effects in children as a result of pre-natal exposure such as motor skill and social emotional developmental impairment [13].

1.5 Animal Research on $PM_{2.5}$ Effects

A variety of animal models are used to gain a more complete understanding of how $PM_{2.5}$ exposure may affect development and health across various species. Studies involving $PM_{2.5}$ exposure in mice demonstrated increased levels immune and inflammatory cells in lung tissue, alveolar damage, intracellular edemas, microvilli, and lamellar bodies in lung tissue[14]. behavioral and developmental abnormalities were observed in monkeys exposed to high levels of $PM_{2.5}$. Increased levels of aggression and fighting in monkeys in air with higher pollution levels were also observed, while another recorded adverse impacts to monkeys' immune systems due to increased $PM_{2.5}$ levels[15]. Additionally, a study of domesticated dogs in Mexico City proposed evidence dogs exposed to severe air pollution exhibited much higher levels of COX2 expression, an enzyme important in immune and inflammatory response in their brains [16].

1.6 Zebrafish as a model organism

The effects of $PM_{2.5}$ were analyzed in zebrafish development in this study because of their use as model organisms in scientific studies. Specifically, zebrafish are recognized as model organisms because they show high genetic homology with humans, develop rapidly post-fertilization (growing as much in one day as a human does in one month), and are nearly

transparent, allowing for easy examination of internal structure and organogenesis[17]. Humans and zebrafish share 70 percent of the same genes and 84 percent of human genes known to be associated with human disease have a counterpart in zebrafish[18]. Other benefits of zebrafish developmental analysis include external fertilization and development, small size allowing them to fit into 96-well plates during embryogenesis, and cost-effective growth[19]. Zebrafish are also useful for behavioral analysis as they can be used to study movement changes with light dark transitions at early points during development to identify developmental toxicants[20].

1.7 Previous zebrafish studies

Previous $PM_{2.5}$ studies that used zebrafish for research have presented evidence of many developmental abnormalities in embryonic zebrafish linked to environmental $PM_{2.5}$ exposure. the resultant embryonic toxicity and the addition of $PM_{2.5}$ into developing zebrafish's water led to increased mortality and inhibited hatching rate of the fish in a dose-dependent manner[21]. Other morphological abnormalities are that as irregular development and increased autophagic cell accumulation of heart, liver, intestines, and muscle tissue, a similar effect to those found in the studies of mice[22]. Other observations include increased expression of genes related to inflammation and autophagy, reduced body length and increased ROS levels, as well as a reduction in locomotor capacity in larvae at high doses of $PM_{2.5}$.

While these studies have delineated many of the developmental and behavioral effects in these animals due to $PM_{2.5}$ exposure, there is a large gap in research concerning both zebrafish strain and $PM_{2.5}$ components used. Specifically, the differences in reaction to particulate matter across wild-type strains of zebrafish are largely unknown, as well as the variance in effect in

larvae behavior due to the physical particles versus chemical components of $PM_{2.5}$. This study aims to address these gaps as mentioned below.

1.8 Goals of this study

This study investigated the developmental effects that environmental $PM_{2.5}$ exhibits on zebrafish in the laboratory. The zebrafish were placed in 24-well plates after fertilization in fish water with varying concentrations of $PM_{2.5}$ solution created prior to the experiment as whole particle suspensions (WPS) and soluble fractions. I hypothesized that as the concentration of $PM_{2.5}$ increased in each treatment group, the total amount of movement in each group would also increase. I also hypothesized that treatment raised in the WPS would have more statistically significant differences in total movement than those raised in the soluble fraction. The effects of each $PM_{2.5}$ sample were measured by using the Viewpoint Zebralab instrument that employs video-tracking software and light/dark modulations to track each fish's behavior. Because of zebrafish and human's high level of genetic homology, the results of this study can help support research that investigates the possible link between specific $PM_{2.5}$ compounds and developmental effects in humans.

METHODS AND MATERIALS

2.1 Source of $PM_{2.5}$:

In order to create reproducible and consistent exposures in this experiment when establishing the model, the National Institute of Standards and Technology (NIST) standard reference material "Fine Atmospheric Particulate Matter" (SRM 2786) was utilized. Storage for SRM 2786 was followed based on NIST recommendations (away from light at room temperature). The SRM is a well-characterized particulate matter sample that while defined as "fine" by the NIST contains particles that are < 4 μm in diameter, thus containing $PM_{2.5}$ as well as $PM_{4\text{-}2.5}$. This is the smallest size fraction of PM that is commercially available. For consistency in the rest of this thesis I will refer to SRM 2786 as SRM.

Re-suspension of $PM_{2.5}$: To begin, 14.0 mg of DMSO was weighed out and placed into an amber vial. Next, 350 μL of SRM was then added to the vial. The mixture was then vortexed for 5 seconds and sonicated for 1 minute in a waterbath sonicator (60 Hz) to ensure proper mixing.

2.2 Fractions of $PM_{2.5}$

2.2a Whole Particle Suspension (WPS)

I then created a WPS to create dilutions of the DMSO and SRM mixture, 50 mL conical tubes were prepared and labeled at concentrations of 0, 12.5, 25, 50, 100, and 200 μg/mL. The conical tube labeled 0 μg/mL was prepared with a volume of 150 μL of DMSO and 29.85 mL of fish water (pH 70.-7.6, 340 parts per million Instant Ocean). The 50 mL conical tubes labeled

12.5, 25, 50, and 100 µg/mL were prepared with volumes of 75 µL of DMSO and 15 mL of fish water each. Finally, the 50 mL conical tube labeled 200 µg/mL was prepared with a volume of 0 µL of DMSO and 30.85 mL of fish water. Once the tubes were prepared the re-suspended SRM (section 2.1) was added to the 200 µg/mL tube the solution of SRM, DMSO, and fish water was sonicated for 1 min (60 Hz) and vortexed for 5 seconds. Following this a serial dilution was performed for all the remaining concentrations with identical sonication and vortexing steps for each solution (Figure 1). The final concentration of DMSO in all tubes was 0.5%.

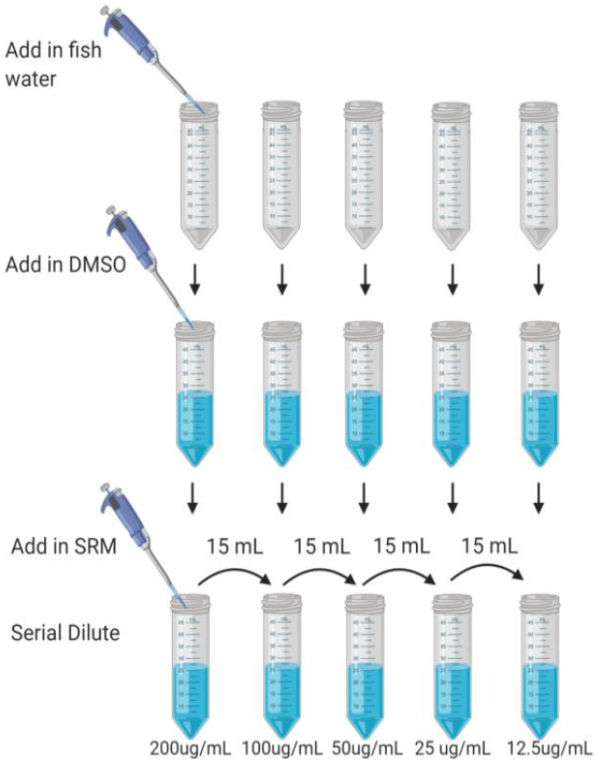


Figure 1: Diagram showing the steps to creating a serial dilution. After the addition of fish water and DMSO, the SRM/DMSO mixture was added to the 200 µg/mL conical tube. Prior to each step in the serial dilution, samples were sonicated and vortexed before each extraction to ensure optimal mixture of soluble and insoluble fractions for removal by pipetting.

2.3b Soluble Fraction

The WPS contained both the soluble and insoluble fractions of the SRM in DMSO. In order to separate these fractions, an aliquot of the 200 µg/mL WPS was centrifuged for 5 minutes at 13 Gs in a 1.5 mL centrifuge tube (Figure 2). Due to and gravity and the centrifugal force, the more dense, insoluble particles fall to the bottom of the tube, and the remaining soluble fraction rests above as the supernatant. Next, 155 µL of the soluble fraction was then removed from the centrifuge tube and placed in a new 50 mL conical tube labeled 200 µg/mL containing a volume of 0 µL of DMSO and 30.85 mL of fish water to create the 200 µg/mL solution with a final concentration of 0.5% DMSO. Similar to the WPS, 50 mL conical tubes were labeled 12.5, 25, 50, and 100 µg/mL for the soluble fraction were prepared with volumes of 75 µL of DMSO and 15 mL of fish water each. I then preformed a serial dilution to create exposure solutions of 12.5, 25, 50, 100, and 200 µg/mL of SRM in DMSO (Figure 1). This dilution was performed with a new set of conical tubes, excluding the conical tube labeled 0 µg/mL prepared with the WPS as there was no need to recreate this concentration.

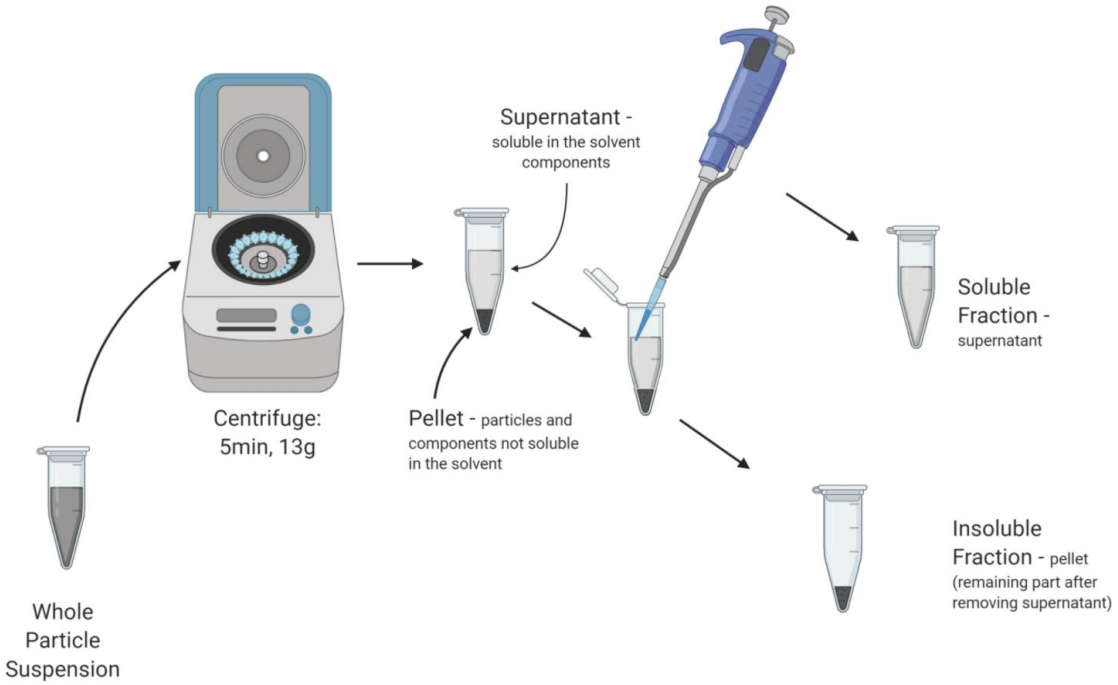


Figure 2: Presentation of the steps taken to separate the soluble and insoluble fractions of a whole particle suspension. The resulting supernatant fluid is the soluble fraction used to create another serial dilution.

2.3 Zebrafish Husbandry

Two wild-type lines of zebrafish (5D and AB) were maintained in a 28 ° Celsius environment on a recirculating system, with a 14-hour light/10 hours dark cycle in Faser Hall at the University of Mississippi. Embryos were collected from adult zebrafish and placed into 24-well plates at 6 hours post fertilization (hpf). Husbandry of the zebrafish was conducted according to University of Mississippi Animal Care and Use Protocols.

2.4 Exposures

At 6 hpf, embryos (n=11/well) were plated and exposed to either the WPS treatment (12.5, 25, 50, 100, 200 µg/mL), soluble fraction treatment (12.5, 25, 50, 100, 200 µg/mL),

vehicle control (0.5% DMSO in fish water), or positive control (200 µg/mL of SRM1649b prepared identically to the 200 µg/mL SRM2786 used for the WPS and soluble fractions). Each treatment and control group contained 33 animals. Each 24-well plate contained 2 wells of each treatment/control, one per each fish line, as demonstrated in Figure 3. Identical plates were exposed on 3 different days to control for differences between plates and days of exposure.

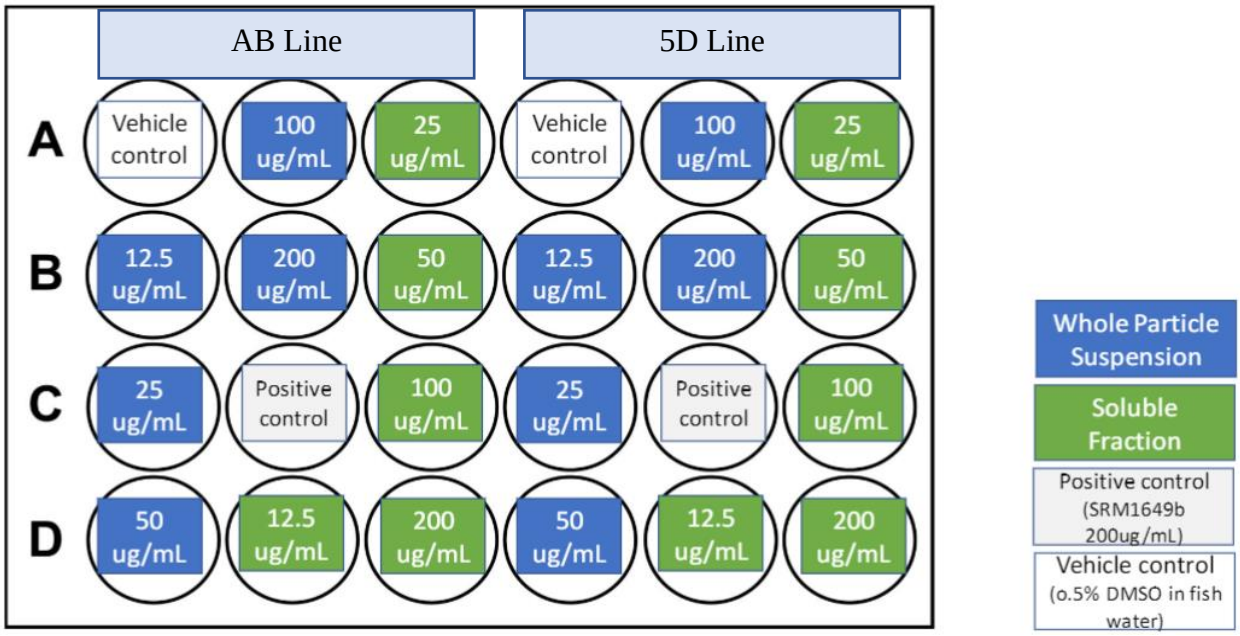


Figure 3: Representation of the layout of each 24-well plate in which the developmental exposures occurred.

2.5 Morphology and mortality

Assessments were made at 24 hpf and 5 days post fertilization (dpf) to determine morphological endpoints and mortality. At 5 dpf, all fish were individually photographed under a dissecting microscope and the pictures were examined for the following: an uninflated swim

bladder, a yolk sac edema, pericardial edema, bent axis, and tail malformation. Mortality/failure to progress past embryonic stage was noted as well. I conducted a blinded examination of each animal, without knowledge of which were treatments and controls. This data analysis is ongoing and will not be included in this thesis, but all animals analyzed for behavior assessments were observed to ensure they were able to freely swim at 5 dpf.

2.6 Behavioral assessments:

At 5 dpf, each embryo was transferred from a 24-well plate to a 96-well plate prior to assessment with a single larva in each well. Behavior was assessed at 5 dpf by analyzing swimming distance during repeated light dark transitions following a 5-minute acclimation period. All analyses were conducted using a Viewpoint Zebralab instrument that employs video-tracking software and light/dark modulations to track zebrafish behaviors. Each test contained three different phases: Light 1, Dark, then Light 2 (in that order). During Light 1 and 2 phases, fish were exposed to light in the Zebralab instrument, and their amount of movement was recorded. This same process was repeated in the Dark phase, however the light in the instrument was off, and the fishes' behaviors were recorded in darkness with each phase lasting for 5 minutes.

2.7 Statistical analysis:

All analyses were conducted in Excel and SigmaPlot (Version 14). Averages ± standard error means (SEM) of total swimming distance were calculated for each treatment and control group for each light phase. Animals were removed that recorded 0 mm of movement across all three phases as these findings were likely due to damage to the larvae in the well plate transfer

process. Differences in total swimming movement in each phase between all groups was determined using one- or two-way analysis of variance (ANOVAs) with pairwise multiple comparison procedures. The two independent variables measured were the preparation of SRM used (WPS and soluble fraction) and concentration of SRM. Statistical significance was set at a p-value ≤ 0.05.

RESULTS

3.1 Zebrafish sample size

Total movement during each of three light phases (Light 1, Dark, and Light 2) was measured for each larval zebrafish in the Viewpoint instruments. Average movement in mm was determined for each exposure group in the AB and 5D lines and comparisons were made between treatment and control groups, phases, fractions of SRM, and zebrafish strains. Table 1 lists the number of animals assessed for each group.

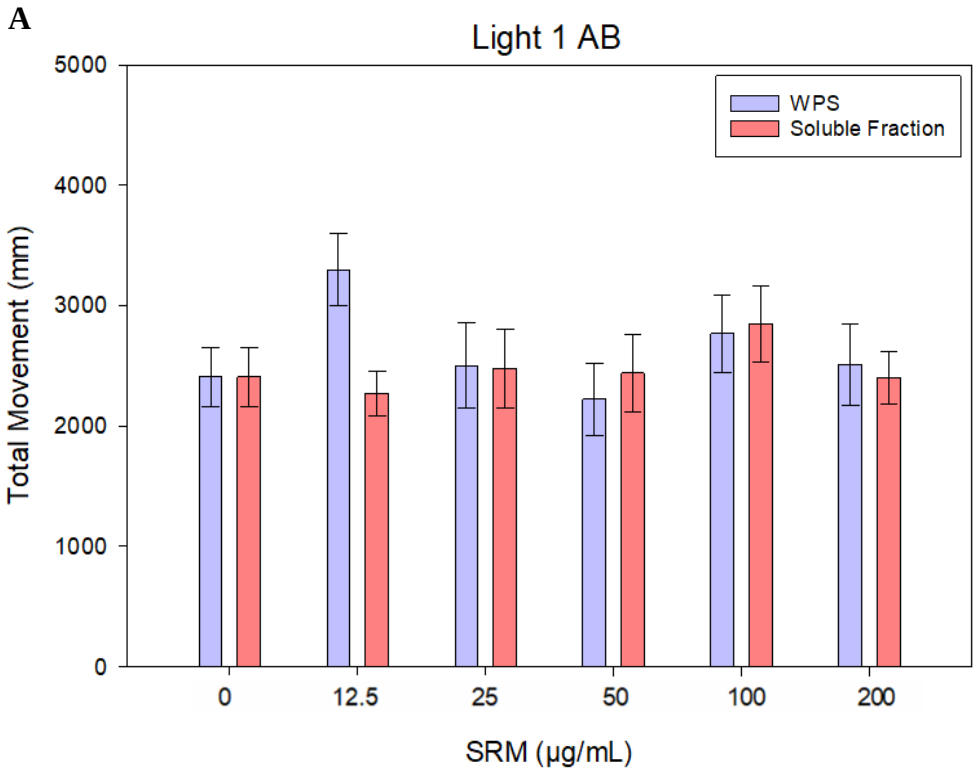
Table 1: The number of zebrafish assessed in behavior analysis for both

		Whole Particle Suspension					**Soluble Fraction**				
SRM (µg/mL)	**0**	**12.5**	**25**	**50**	**100**	**200**	**12.5**	**25**	**50**	**100**	**200**
AB Strain	33	34	35	36	35	32	35	35	35	36	32
5D Strain	35	35	36	35	31	34	32	35	34	34	36

3.2 Comparison between Light Phases in Each Fish Line

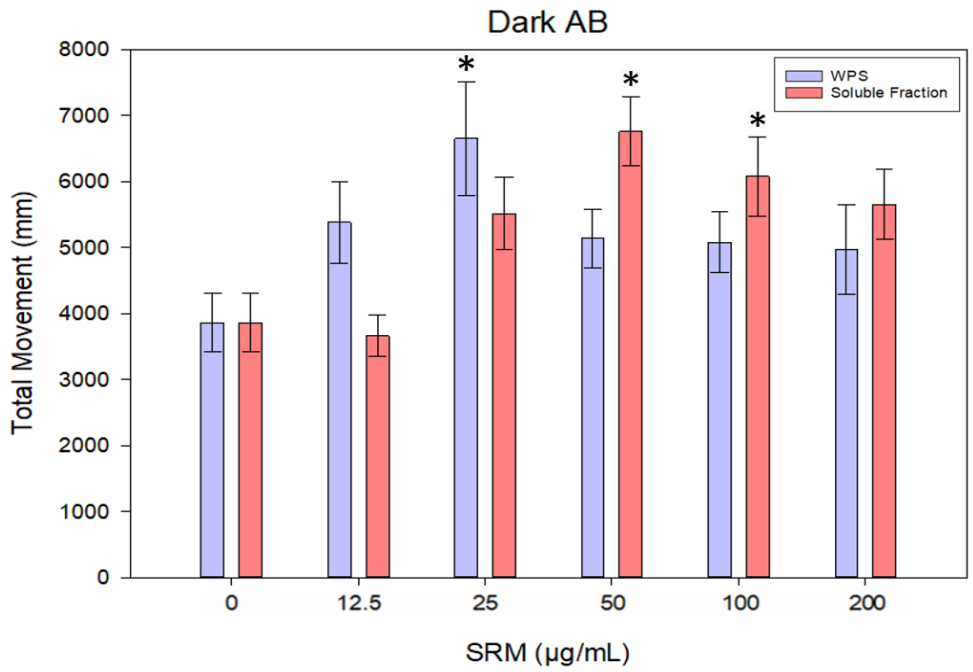
Significant differences between the DMSO control and each exposure concentration of SRM are denoted by an asterisk for the AB (Figure 4) and the 5D (Figure 5) lines. For Light Phase 1 in the AB line (Figure 4A) and the 5D Line (Figure 5A) there were no signficant

differences observed between the treatment and control groups. Most significant differences occurred in the Dark Phase for both the AB line and the 5D Line. However, the treatment groups in Dark 5D conditon (Figure 5B) contained seven instances of significant differences from the DMSO control, compared to the three significant differences in the Dark AB condition (Figure

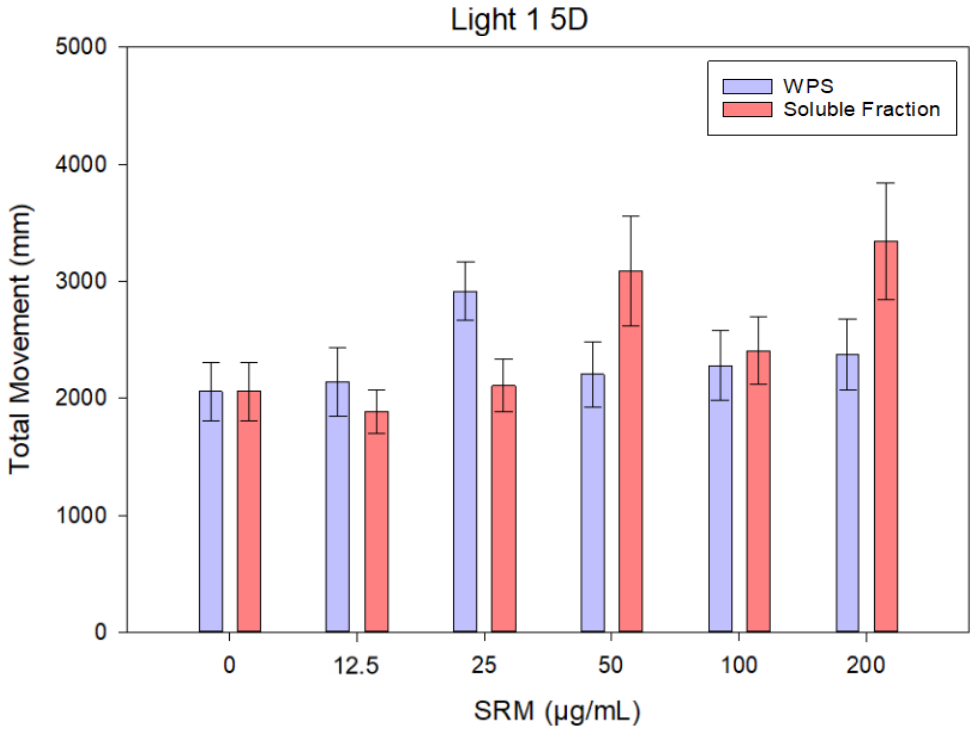


4B). As for the Light 2 Phase, the 5D line (Figure 5C) contained one significant difference in the 25 µg/mL treatment group, while the AB line (Figure 4C) contained no signficant differences between the treatment and control groups.

B


Dark AB
WPS
Soluble Fraction
Total Movement (mm)
8000
7000
6000
5000
4000
3000
2000
1000
0
*
*
*
0
12.5
25
50
100
200
SRM (µg/mL)

A


Light 1 5D
WPS
Soluble Fraction
Total Movement (mm)
5000
4000
3000
2000
1000
0
0
12.5
25
50
100
200
SRM (µg/mL)

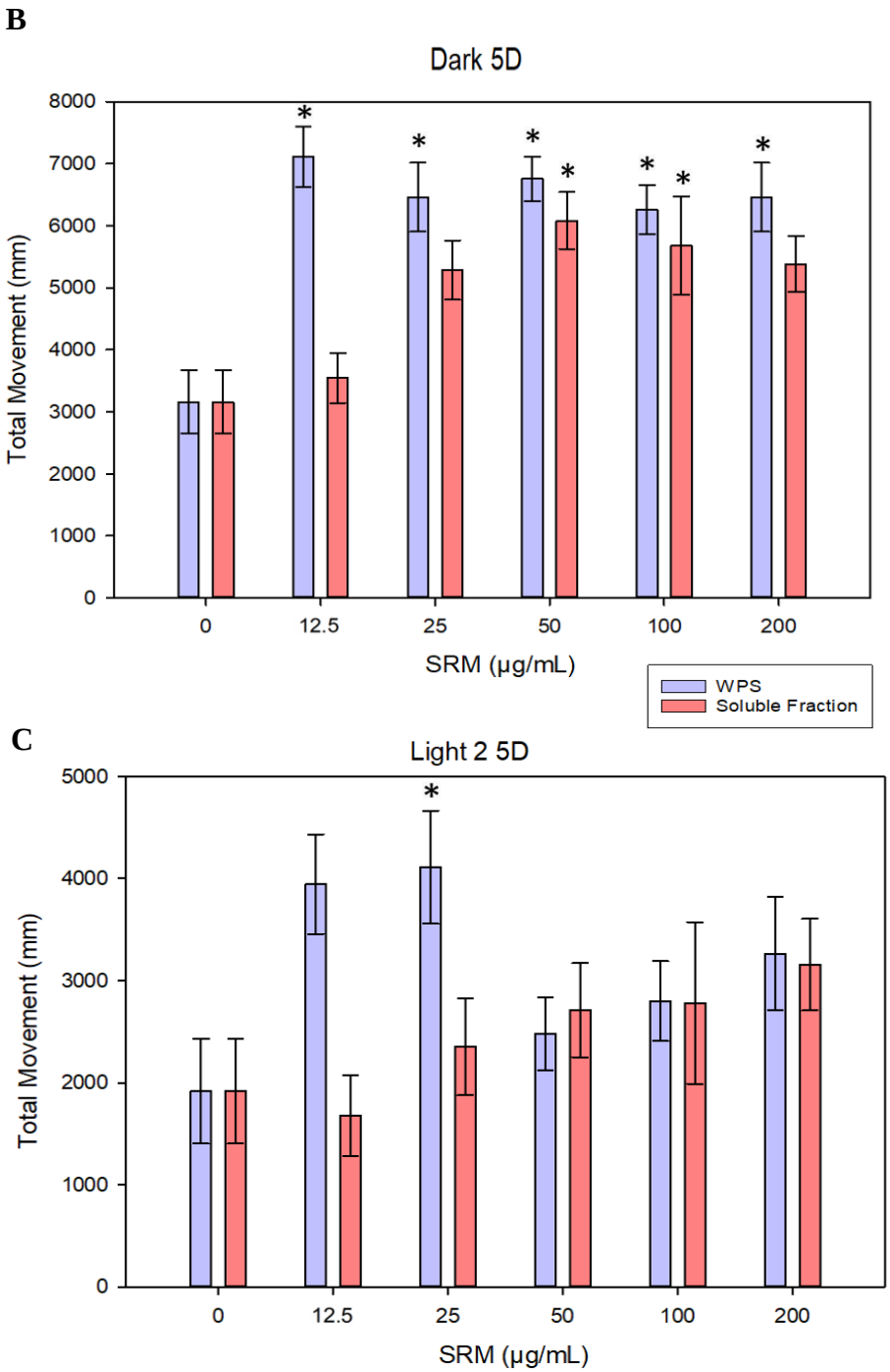


Figure 5: Total movement by zebrafish larvae of the 5D line in each of the three light phases exposed to different SRM concentrations. Averages ± SEM for total movement during the Light 1 (A), Dark (B), and Light 2 (C) phases for each SRM concentration (0, 12.5, 25, 50, 100, & 200 µg/mL) with animals per group listed in Table 1. Embryos exposed to the whole particle suspension are represented in purple, while those exposed to the soluble fraction only are represented in red. Each * denotes a statistically significant difference ($P \leq 0.05$) between the DMSO control and the experimental group based on a two-way ANOVA.

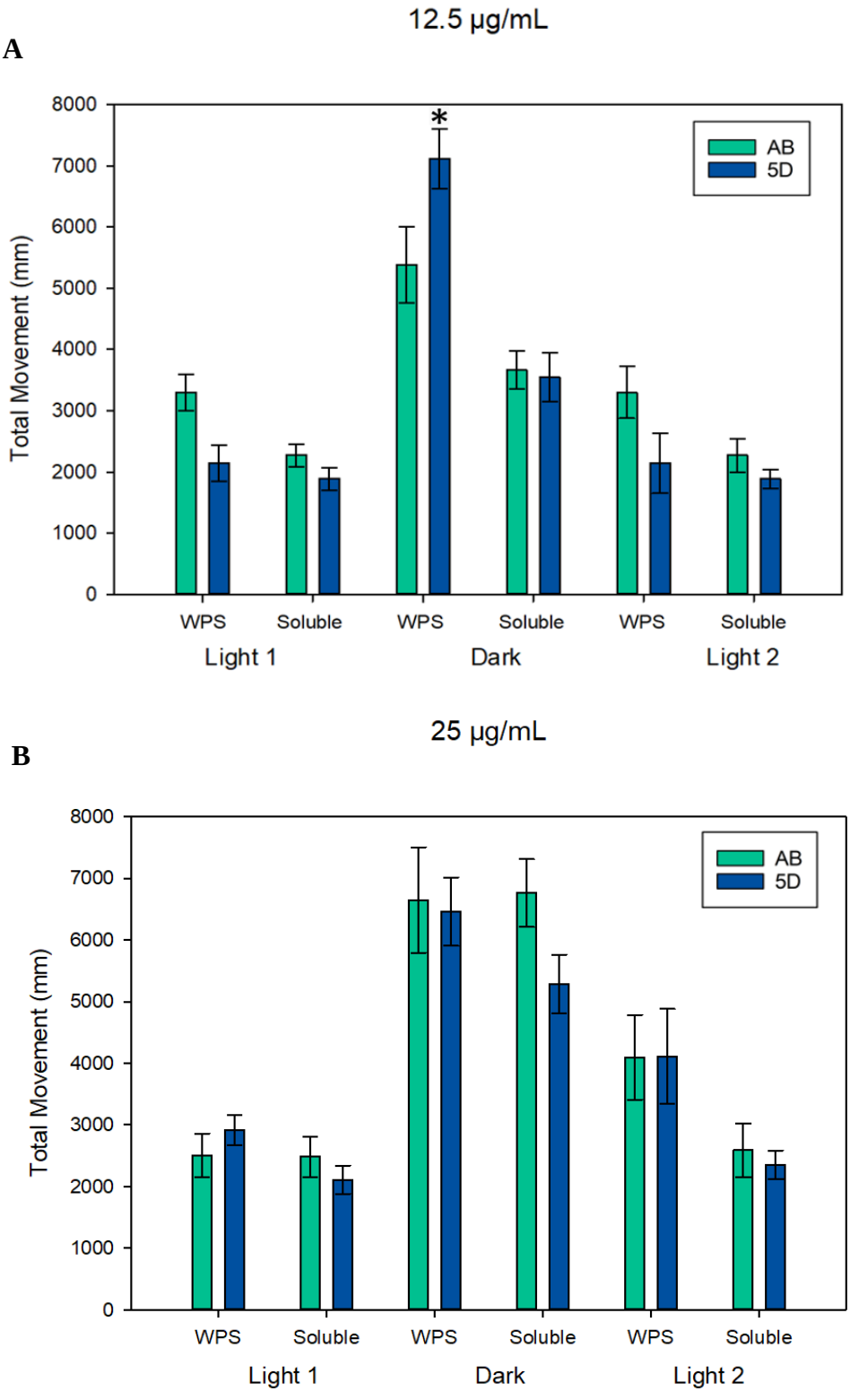
3.3 Comparison between Soluble Fraction and Whole Particle Suspension

Overall, the amount of zebrafish movement in the soluble fractions and whole particle suspensions tended to be similar across each concentration, regardless of strain, for all light phases. However, for the Dark and Light 2 phases in the 5D line there were statistiscally signficant differences between the whole particle and soluble fractions at 12.5 μg/mL. These differences were not observed in the AB line. Overall, the fish exposed to the WPS solutions had more instances of statistically significant differences in comparison to their respective DMSO group than those raised in the soluble fraction.

3.4 Comparison of Zebrafish Strain

Although the amount of movement in each strain throughout all three phases remained relatively similar, there were eight significant differences noted in the treatment groups and their respective control group in the 5D line, while only three were recorded in the AB line. These differences are especially notable in the 12.5 ug/mL exposures to SRM (Figure 6a), in which there was a significant difference between the AB and 5D lines in the dark phase for the WPS. This was not observed in the soluble fraction or in the other light phases. There were no significant differences recorded in the 25 μg/mL condition (Figure 6b) or the 50 μg/mL condition (Figure 6c). Overall, there was a trend that the AB line had higher average movement compared to the 5D line in Figures 6 and 7. Similarly, there were also significant differences in the two lines in the Light 1 soluble fraction of the 200 μg/mL concentration (Figure 7b), and in the WPS

of the 100 µg/mL concentration (Figure 7a), neither of which were observed in other light phases.



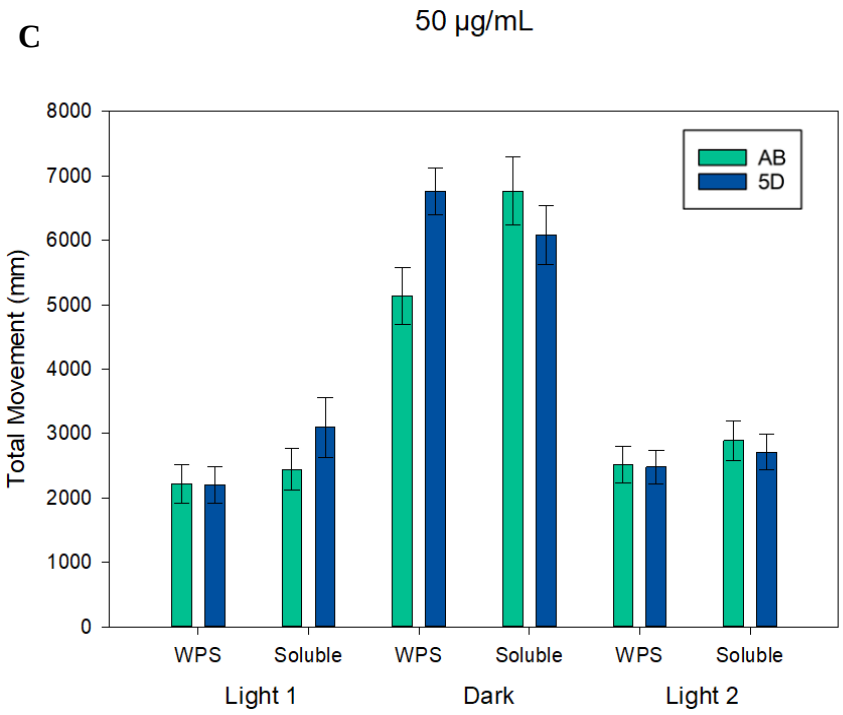
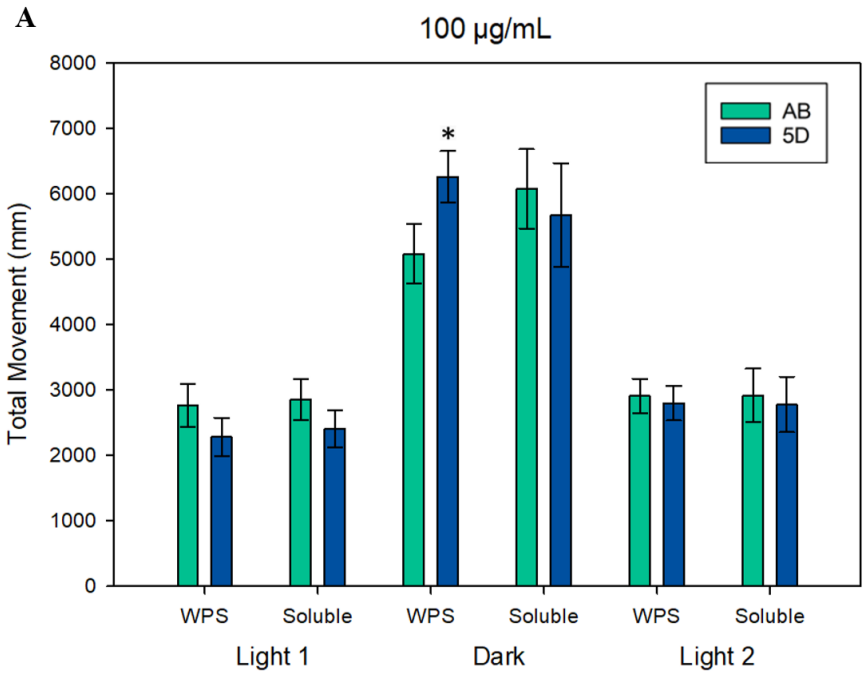


Figure 6: Total movement by zebrafish larvae of both the AB and 5D lines in each of the three light phases raised in either WPS or soluble fraction at concentrations of 12.5, 25, and 50 µg/mL. Averages ± SEM for total movement during the Light 1 (A), Dark (B), and Light 2 (C) phases for each condition with animals per group listed in Table 1. Larvae of the AB line are represented in green, while members of the 5D line are represented in blue. Each * denotes a statistically significant difference ($P \leq 0.05$) between the DMSO control and the experimental group based on a two-way ANOVA.



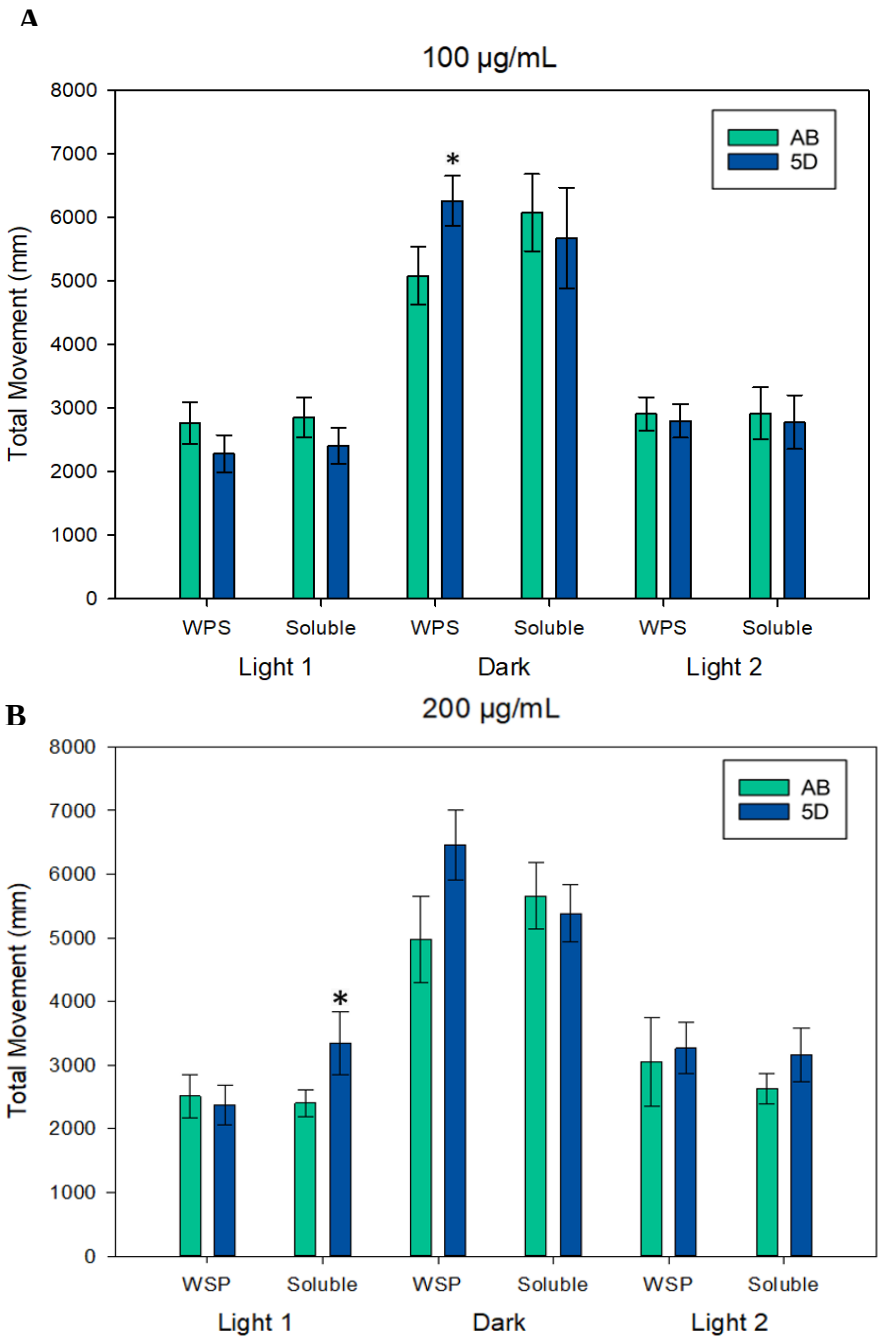


Figure 7: Total movement by zebrafish larvae of both the AB and 5D lines in each of the three light phases raised in either WPS or soluble fraction at concentrations of 100, and 200 µg/mL. Averages ± SEM for total movement during the Light 1 (A), Dark (B), and Light 2 (C) phases for each condition with animals per group listed in Table 1. Larvae of the AB line are represented in green, while members of the 5D line are represented in blue. . Each * denotes a statistically significant difference ($P \leq 0.05$) between the DMSO control and the experimental group based on a two-way ANOVA.

3.5 Comparison Between Exposure Groups Independent of Fish Strain

Comparisons were made between exposure groups considering all fish tested, regardless of the strain, for each light phase. Upon analysis of the two way ANOVA test results, it was noted that across all fish, independent of fish line, there were significant differences during the Light 1 phase between the soluble and whole particle suspensions at 12.5 μg/mL. No other signficant differences were recorded during this phase.

During the Dark phase, there were significant differences between the soluble and whole particle suspensions at 12.5 μg/mL across all fish, independent of fish line as well. However, there were also significant differences between the DMSO groups and all five WPS treatment groups (12.5, 25, 50, 100, & 200 μg/mL), as well as significant differences between the DMSO groups and four soluble treatment groups (25, 50, 100, and 200 μg/mL) across all fish and independent of fish line. It is worth noting that in comparing the WPS and DMSO groups mentioned, the 12.5, 25, and 50 μg/mL groups were very statistically significant ($P<.001$), while the 100 & 200 μg/mL groups had P values of .003 and .002, respectively.

During the Light 2 Phase, there were no significant differences found between the treatment groups and the DMSO groups, however there was a non-signficant positive trend of movement as SRM concentration increased in both the AB and 5D lines during this phase.

3.6 Comparison of Phases

In examining differences between the Light 1, Dark, and Light 2 Phases, it is evident that, on average, movement was highest in every condition during the Dark Phase. Interestingly, the

Light 1 Phase contained no statistically significant differences between the treatment and DMSO groups in either line, but there were differences observed in the Dark Phase for both lines. Finally, the only statistically significant difference between the treatment and DMSO groups observed in the Light 2 Phase was between 25 μg/mL and the DMSO group of the 5D line.

DISCUSSION

4.1 Differences in Behavioral Response Following $PM_{2.5}$ Exposure by Condition

To date, we are unaware of another study in which the AB and 5D lines of zebrafish have been compared in their response to $PM_{2.5}$. Therefore, it was difficult to predict the difference in behavioral responses between each line and to the SRM used throughout this experiment.

As mentioned in section 2.2, the WPS and soluble fractions are similar in that both contain DMSO-soluble chemicals. The soluble fraction does not contain many DMSO-insoluble compounds or the physical particles since they have been forced to the bottom by centrifugation. The WPS contains relatively large, DMSO-insoluble particles of $PM_{2.5}$ as well as the same soluble chemicals.

In comparing the whole particle suspension and soluble fraction throughout this experiment, it is evident that there are very few instances of significant differences between either line's overall amount of movement. The only times in which these were recorded were in comparison of the 12.5 μg/mL WPS and soluble fraction, found in both the Dark and Light 2 Phases. Because this difference was only significant in two of the eighteen conditions, it appears that there are more factors driving behavioral effects than the non-soluble particles alone. If the particles alone were solely responsible for the change in behavior, we would most likely have seen very different levels of behavior between the WPS and soluble fraction conditions in their absence.

In one study investigating the effects of silica nanoparticles (SiNPs) in AB line fish, a specific particle that can be found in $PM_{2.5}$, researchers found that lower concentrations of exposure (25 and 50 µg/mL SiNPs) caused substantial hyperactivity, while higher doses (100 and 200 µg/mL SiNPs) were correlated with remarkable hypoactivity in Dark phases[23]. The results of this study also showed substantial hyperactivity in lower doses of $PM_{2.5}$ exposure, as shown in Figures 5b, 6b, 6c, and 7a. However, our results differ in that higher doses of $PM_{2.5}$ exposure did not lead to hypoactivity in any condition, regardless of line.

Another experiment examined zebrafish response to PAHs by introducing developing 5D line fish to ten of the most common PAHs found in $PM_{2.5,}$ a solution referred to as SM10[24]. Fish in the experimental condition showed significantly more movement over the course of the experiment in comparison to the DMSO control group, likely as a result of long-term exposure to SM10 during development. The results of our study are consistent with these findings in certain conditions as shown in Figures 4b, 5b, 5c, 6a, 7a, and 7b.

I originally hypothesized that treatment raised in the WPS would have more statistically significant differences in total movement than those raised in the soluble fraction. In reviewing the results of the ANOVA analysis and Figures 4 and 5, it is clear that the embryos raised in the WPS solution had more instances of significant differences from the control group than those raised in the soluble fraction, so I was able to accept this hypothesis. I also hypothesized that as the concentration of $PM_{2.5}$ increased in each treatment group, the total amount of movement in each group would also increase. However, as there was little data in support of this as seen in Figures 4 & 5, I was unable to accept this hypothesis.

4.2 Differences in Reaction to $PM_{2.5}$ by Line

It is beyond the scope of this experiment to be able to definitively explain the reasons that the 5D line contained more statistically significant differences from the DMSO control than the AB line (as shown in Figures 5 and 6). However, it does appear that the fish of the AB line had less significant and consistent responses to the 5D line. This is observed throughout Figures 6 and 7, in which we see many more significances from the control group in the 5D than AB line.

It is possible that the genetic differences in these two lines cause different interactions between the zebrafish and the $PM_{2.5}$. For example, the AB and 5D lines of zebrafish respond differently to developmental exposure to nicotine and imidacloprid (a prototypic pesticide), in that the 5D strain of zebrafish were more sensitive to both experimental compounds than the AB strain[25]. This result is similar to our own in that the 5D strain showed more consistent and significant responses to developmental $PM_{2.5}$ exposure than the AB strain, as shown throughout Figures 6 and 7.

Another cited differences in dopamine response across the two strains across light and dark conditions[26]. In this experiment, both lines were exposed to either SCH-23390 (a D_1 receptor antagonist) or haloperidol (a D_2 receptor antagonist). In the end, the 5D line was less sensitive to the effects of SCH-23390 during the dark phase (differing from our experimental group) but was more sensitive to haloperidol than the AB line (similar to our results). Other possible explanations include inbreeding in one or both of the lines, differences in how the lines were crossed, and more.

4.3 Future Direction

To increase our understanding of the ways in which $PM_{2.5}$ exposure effects the AB and 5D lines of developing zebrafish differently, further investigation into the developmental outcomes in these animals must be completed. Examining differences in developmental abnormalities could yield more indication as to the differences between these two lines, such as the frequency of pericardial and yolk sac edemas and the inflation of individual's swim bladders, and more. Other research that could be useful in identifying harmful effects of $PM_{2.5}$ in these lines is by exposing them to specific chemicals commonly found in $PM_{2.5}$, such as specific metals or PAHs. There is much more work to be done in this field to fully understand the mechanisms behind particulate matter's effects on zebrafish, but these opportunities can help us to understand the ways more fully in which $PM_{2.5}$ interacts with our own bodies as well.

LIST OF REFERENCES

1. US EPA, O. (2014, April 9). Criteria Air Pollutants [Other Policies and Guidance]. US EPA. https://www.epa.gov/criteria-air-pollutants

2. Adams, K., Greenbaum, D. S., Shaikh, R., van Erp, A. M., & Russell, A. G. (2015). Particulate matter components, sources, and health: Systematic approaches to testing effects. Journal of the Air & Waste Management Association, 65(5), 544–558. https://doi.org/10.1080/10962247.2014.1001884

3. Nelin, T. D., Joseph, A. M., Gorr, M. W., & Wold, L. E. (2012). Direct and Indirect Effects of PM on the Cardiovascular System. Toxicology Letters, 208(3), 293–299. https://doi.org/10.1016/j.toxlet.2011.11.008

4. Hsiao, W. L., Mo, Z. Y., Fang, M., Shi, X. M., & Wang, F. (2000). Cytotoxicity of PM(2.5) and PM(2.5—10) ambient air pollutants assessed by the MTT and the Comet assays. Mutation Research, 471(1–2), 45–55. https://doi.org/10.1016/s1383-5718(00)00116-9

5. Xing, Y.-F., Xu, Y.-H., Shi, M.-H., & Lian, Y.-X. (2016). The impact of PM2.5 on the human respiratory system. Journal of Thoracic Disease, 8(1), E69–E74. https://doi.org/10.3978/j.issn.2072-1439.2016.01.19

6. Du, Y., Xu, X., Chu, M., Guo, Y., & Wang, J. (2016). Air particulate matter and cardiovascular disease: The epidemiological, biomedical and clinical evidence. Journal of Thoracic Disease, 8(1), E8–E19. https://doi.org/10.3978/j.issn.2072-1439.2015.11.37

7. Fu, P., Guo, X., Cheung, F. M. H., & Yung, K. K. L. (2019). The association between PM2.5 exposure and neurological disorders: A systematic review and meta-analysis. The Science of the Total Environment, 655, 1240–1248. https://doi.org/10.1016/j.scitotenv.2018.11.218

8. Lee, C.-W., Vo, T. T. T., Wu, C.-Z., Chi, M.-C., Lin, C.-M., Fang, M.-L., & Lee, I.-T. (2020). The Inducible Role of Ambient Particulate Matter in Cancer Progression via Oxidative Stress-Mediated Reactive Oxygen Species Pathways: A Recent Perception. Cancers, 12(9), 2505. https://doi.org/10.3390/cancers12092505

9. Alman, B. L., Stingone, J. A., Yazdy, M., Botto, L. D., Desrosiers, T. A., Pruitt, S., Herring, A. H., Langlois, P. H., Nembhard, W. N., Shaw, G. M., Olshan, A. F., Luben, T. J., & National Birth Defects Prevention Study. (2019). Associations between PM2.5 and risk of preterm birth among liveborn infants. Annals of Epidemiology, 39, 46-53.e2. https://doi.org/10.1016/j.annepidem.2019.09.008

10. Zhang, T., Zheng, X., Wang, X., Zhao, H., Wang, T., Zhang, H., Li, W., Shen, H., & Yu, L. (2018). Maternal Exposure to PM2.5 during Pregnancy Induces Impaired Development of Cerebral Cortex in Mice Offspring. International Journal of Molecular Sciences, 19(1). https://doi.org/10.3390/ijms19010257

11. Ebisu, K., & Bell, M. L. (2012). Airborne PM2.5 chemical components and low birth weight in the northeastern and mid-Atlantic regions of the United States. Environmental Health Perspectives, 120(12), 1746–1752. https://doi.org/10.1289/ehp.1104763

12. Negative birth outcomes linked to air pollution exposure early in pregnancy, study finds. (n.d.). ScienceDaily. Retrieved March 30, 2021, from https://www.sciencedaily.com/releases/2017/07/170727102907.htm

13. Kao, C.-C., Chen, C.-C., Avelino, J. L., Cortez, M. P., Tayo, L. L., Lin, Y.-H., Tsai, M.-H., Lin, C.-W., Hsu, Y.-C., Hsieh, L.-T., Lin, C., Wang, L.-C., Yu, K.-L. J., & Chao, H.-R. (2019). Infants' Neurodevelopmental Effects of PM2.5 and Persistent Organohalogen Pollutants Exposure in Southern Taiwan. Aerosol and Air Quality Research, 19(12), 2793–2803. https://doi.org/10.4209/aaqr.2019.10.0550

14. Yang, J., Chen, Y., Yu, Z., Ding, H., & Ma, Z. (2019). The influence of PM2.5 on lung injury and cytokines in mice. Experimental and Therapeutic Medicine, 18(4), 2503–2511. https://doi.org/10.3892/etm.2019.7839

15. Xu, A., Liu, C., Wan, Y., Bai, Y., & Li, Z. (2021). Monkeys fight more in polluted air. Scientific Reports, 11(1), 654. https://doi.org/10.1038/s41598-020-80002-z

16. Calderón-Garcidueñas, L., Reed, W., Maronpot, R. R., Henríquez-Roldán, C., Delgado-Chavez, R., Calderón-Garcidueñas, A., Dragustinovis, I., Franco-Lira, M., Aragón-Flores, M., Solt, A. C., Altenburg, M., Torres-Jardón, R., & Swenberg, J. A. (2004). Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. Toxicologic Pathology, 32(6), 650–658. https://doi.org/10.1080/01926230490520232

17. Horzmann, K. A., & Freeman, J. L. (2018). Making Waves: New Developments in Toxicology With the Zebrafish. Toxicological Sciences, 163(1), 5–12. https://doi.org/10.1093/toxsci/kfy044

18. Gilbert, S. (2021, March 28). Zebrafish help researchers study human genes | Penn State University. https://news.psu.edu/story/418819/2016/07/28/research/zebrafish-help-researchers-study-human-genes

19. Spitsbergen, J. M., & Kent, M. L. (2003). The State of the Art of the Zebrafish Model for Toxicology and Toxicologic Pathology Research—Advantages and Current Limitations. Toxicologic Pathology, 31(Suppl), 62–87. https://pubmed.ncbi.nlm.nih.gov/12597434/

20. Bailey, J., Oliveri, A., & Levin, E. D. (2013). Zebrafish Model Systems for Developmental Neurobehavioral Toxicology. Birth Defects Research. Part C, Embryo Today : Reviews, 99(1), 14–23. https://doi.org/10.1002/bdrc.21027

21. Duan, J., Hu, H., Zhang, Y., Feng, L., Shi, Y., Miller, M. R., & Sun, Z. (2017). Multi-organ toxicity induced by fine particulate matter PM2.5 in zebrafish (Danio rerio) model. Chemosphere, 180, 24–32. https://doi.org/10.1016/j.chemosphere.2017.04.013

22. Zhang, Y., Li, S., Li, J., Han, L., He, Q., Wang, R., Wang, X., & Liu, K. (2018). Developmental toxicity induced by PM2.5 through endoplasmic reticulum stress and autophagy pathway in zebrafish embryos. Chemosphere, 197, 611–621. https://doi.org/10.1016/j.chemosphere.2018.01.092

23. Duan, J., Yu, Y., Shi, H., Tian, L., Guo, C., Huang, P., Zhou, X., Peng, S., & Sun, Z. (2013). Toxic effects of silica nanoparticles on zebrafish embryos and larvae. PloS One, 8(9), e74606. https://doi.org/10.1371/journal.pone.0074606

24. Geier, M., & Tanguay, R. (n.d.). Systematic developmental neurotoxicity assessment of a representative PAH Superfund mixture using zebrafish—ScienceDirect. Retrieved March 28, 2021, from https://www.sciencedirect.com/science/article/pii/S0041008X18301212

25. Crosby, E. B., Bailey, J. M., Oliveri, A. N., & Levin, E. D. (2015). Neurobehavioral Impairments Caused by Developmental Imidacloprid Exposure in Zebrafish. Neurotoxicology and Teratology, 49, 81–90. https://doi.org/10.1016/j.ntt.2015.04.006

26. Oliveri, A. N., & Levin, E. D. (2019). Dopamine D1 and D2 Receptor Antagonism During Development Alters Later Behavior in Zebrafish. Behavioural Brain Research, 356, 250–256. https://doi.org/10.1016/j.bbr.2018.08.028

27. Roper, Courtney, and Robert L. Tanguay. “Zebrafish as a Model for Developmental Biology and Toxicology.” Handbook of Developmental Neurotoxicology, 2018, pp. 143–151, www.sciencedirect.com/science/article/pii/B9780128094051000122, 10.1016/b978-0-12-809405-1.00012-2. Accessed 23 Apr. 2021.