International Journal of Scientific Research in Biological Sciences
Vol.9, Issue.5, pp.25-29, October (2022)

Research Paper

E-ISSN: 2347-7520

The Effect of Secondhand and Thirdhand E-cigarette Vapor on the Geotaxis and Olfaction of Larval and Adult *Drosophila melanogaster*

Srilekha Mamidala

Department of Biology, Garnet Valley High School, Glen Mills, Pennsylvania, USA

*Corresponding Author: srimamidala11@hotmail.com, Tel.: +01 610 836 1026

Available online at: www.isroset.org

Received: 31/Aug/2022, Accepted: 30/Sept/2022, Online: 31/Oct/2022

***Abstract*—** Rapidly increasing in popularity, e-cigarettes have proved to be detrimental to human health. Appealing most to youth aged 12-17, e-cigarettes have been the cause of thousands of cases of lung-related illnesses around the world. Adequately addressing this situation requires a complete understanding of the effects of e-cigarettes. This study examines how secondhand and thirdhand exposure to e-cigarette vapor affects youth and adult populations via the model organism *Drosophila melanogaster*. This experimental study analyzed groups of 6-10 flies that were exposed to e-cigarette vapor once a day for 8 consecutive days. The experiment analyzed the activity and behavior of the flies and showed that both secondhand and thirdhand exposure to e-cig vapor affects both youth and adult populations negatively through the assessments of geotaxis and olfactory perception. Data from the flies exposed to secondhand and thirdhand vapor were compared to data from flies not exposed to vapor. The flies exhibited both a lower average climb height and reduced ability to smell fruit placed in the environment. Additionally, it was observed that youth were affected more severely than adults, and that thirdhand exposure was almost as harmful as secondhand exposure. The different types of exposure presented to populations in different stages of life prove to have a visible effect on the perceptionary strength of the flies, which may translate to effects experienced by humans undergoing the same types of exposure.

Keywords— E-cigarette, vape, olfaction, geotaxis, perception

I. INTRODUCTION

E-cigarette use has recently risen significantly, despite rising health concerns: chemicals both inhaled into the body and exhaled into the environment can harm the throat and lungs. Currently, over 11% of adults and 20.8% of teens have been reported to vape, but these numbers are increasing as thousands of new cases of vaping-related diseases are being reported, creating an epidemic especially among youth aged 12-17 [1][2]. Alarmingly, 20% of high school students and 5% of middle school students have reported using e-cigarettes [1], potentially affecting 4.7 million students. The marketing of flavors such as mango and peppermint directly targeting this age demographic have led many, especially teens, to become addicted to e-cigarettes, unaware of the consequences caused by chemicals in vape juice such as vegetable glycerin, propylene glycol, and nicotine [3]. Nicotine's tendency to become addictive while also introducing various respiratory and neurological problems [4]. The Centers for Disease Control and Prevention has reported over 2,000 lung illnesses stemming from EVALI (E-cigarette or Vaping product use-Associated Lung Injury) that peaked in September 2019, an outbreak of serious injury via e-cigarettes predominantly affecting teens [2][3][5]. These cases have led to serious lung damage, cancer, and in some cases death.

As e-cigarettes have been introduced into the market fairly recently, research on their effects on the body has not been as extensive as traditional cigarettes. In order to gain a complete understanding, it is essential to know how e-cigarettes affect people of different ages and how it impacts different parts of the body. This study may combat skyrocketing use by creating a greater need for regulations, while also possibly declaring certain chemicals used in e-cigarettes illegal due to their harmful effects on the human body.

Previous work on e-cigarettes has disproved the common misconception that the vapor produced is not harmless water vapor but is instead an aerosol that contains heavy metals such as nickel, lead, and cadmium [6][7]. It has also been shown that e-cigarette use during adolescence harms the developing brain and it is more likely to lead to smoking cigarettes and other drugs later in life [8] [9]. From previous e-cigarette epidemics, research has shown that vitamin E acetate, an additive in e-cigarette products, has been strongly linked to spikes in cases [5]. While e-cigarettes are often touted as a way to eventually quit smoking, the FDA has not approved them as an aid to quit regular smoking. There is no decisive evidence proving that e-cigarettes help someone quit [9].

This paper aims to measure and explain the effects of secondhand and thirdhand e-cigarette vapor on larval and adult populations of *Drosophila melanogaster*. The objective of this experiment was to distinguish and compare the severity of effects of e-cigarette vapor on youth and adult populations. If unbridled, the continuation

and expansion of e-cigarette use among youth may lead to severe damage, especially in the brain and lungs, well into adulthood, as well as increasing the likelihood of an e-cigarette user to become a heavy smoker as an adult. Conclusive evidence showing these differences are instrumental in understanding how the chemicals found in e-cigarettes affect developing bodies. The conclusions aim to establish regulations regarding e-cigarettes, as potential toxins found to have a severely adverse effect on both youth and adult populations may be subject to stricter regulation, therefore protecting the well-being of all populations.

II. RELATED WORK

Related work concerning the effects of e-cigarettes on youth and adult populations has mainly focused on the different marketing strategies employed by companies selling e-cigarettes, and their subsequent influence on these populations. It was generally found that exposure to marketing greatly increased the likelihood of a teen to try e-cigarettes, and that increased marketing restrictions would aid in slowing the spread of usage among younger populations [10] [11]. Another study explored the effects of the recent ban of flavored e-cigarettes in the United States via a longitudinal study, concluding that the current policies did not reflect a potential reversion to cigarette use due to the decrease in e-cigarette use due to the ban [12].

III. METHODOLOGY

The experiment was performed with 5 trials. Each trial contained 4 experimental groups: secondhand vapor exposure with larvae flies, thirdhand exposure with larvae flies, secondhand exposure with adult flies, and thirdhand exposure with adult flies. The two control groups for comparison had no exposure to vapor, with one jar with adults only and another with larvae only.

At the start of the experiment, prior to exposure, the geotaxis and olfaction of the flies were recorded. The flies were then exposed to e-cigarette vapor from a vape pen once a day for eight days. Geotaxis and olfaction of the flies was measured again every two days until eight days of exposure, at the conclusion of the experiment.

Fruit flies of the wild type Oregon R strain were purchased from Carolina Biological Supply and were placed in identical jars containing yeast and water for food. Mason jars measuring 7 cm x 7cm x 10 cm were used to house the flies. Two layers of mesh (holes 0.1 in) were attached at the top of the jar instead of a lid to provide breathing air and to prevent escape. The e-cigarette device used was a vape pen manufactured by Smok. The vape pen holds 0.8 mL of liquid. The e-cigarette liquid used was manufactured by Twst Salt E-liquid. The flavor was Pink Punch Lemonade, with a nicotine concentration of 1:60. This flavor was used as it clearly appeals to teenagers and is therefore more representative of an e-cigarette liquid that may be consumed by younger populations. In addition, the vape pen is small and relatively simple to use, making it more likely to be used by younger populations. Active ingredients in the liquid include vegetable glycerin and propylene glycol.

No humans were involved in vaping e-cigarettes for exposure to the fruit flies. E-cigarette vapor was produced from the vape pen by using a suction device that activated the pen similarly to a human mouth via inhalation and exhalation. This caused the e-cigarette to emit vapor from holes on the side. This outwardly emitted vapor was then tilted into the jar. Since these fumes naturally move upward, a narrow cardboard box was placed over the jar to collect the smoke. After all vapor had been emitted, the cardboard box completely enclosed it, thus preventing any vapor from escaping and forcing it to travel down into the jar. Flies were given 10 to 12 puffs of vapor a day, representative of the minimum number of times an e-cigarette user may vape in a day. Therefore, the adverse reactions recorded in this experiment are the lowest possible, and would theoretically increase with more frequent e-cigarette use. Flies receiving secondhand vapor exposure simply received the vapor in its gaseous form. Flies receiving thirdhand vapor exposure were placed in a jar with a sponge at the bottom for absorption of vapor and residue. To prevent these flies from receiving both secondhand and thirdhand exposure and confusing the results of the experiment, the cardboard box did not encapsulate the jar after vapor exposure was done. This way, vapor residue drifted down to be absorbed by the sponges, and the vapor itself was not trapped in the jar, eliminating potential secondhand exposure.

Geotaxis Assay: Geotaxis is defined as an organism's motion in response to gravity. *Drosophila melanogaster* has been known to exhibit a negative geotaxis, meaning they naturally fly upward. Negative geotaxis has also been defined as the measure of the speed of a fly as it climbs back to the top of its environment after being tapped down to the ground, therefore measuring a fly's instinctive ability to attempt escape [13]. To measure geotaxis, the height in centimeters the fruit flies flew at was recorded via markings made on the side of the jar. Markings were made at every cm until the 10 cm height. Heights for each fly were taken separately and averaged to the nearest 0.1 cm. At the start of the experiment, fly counts were recorded so that each fly's height would only be recorded once, albeit through careful observation. Fly counts and averages were performed multiple times to ensure that consistent averages were being achieved.

Olfaction Assay: Olfaction is a chemoreception that enables an organism to smell. *Drosophila melanogaster* contains highly sensitive olfactory bulbs that enable it to identify potential food sources, which helps with the finding of egg-laying sites [14]. Olfaction data was taken by placing a crushed banana on top of a small-holed mesh square at the top of the jar and measuring the percentage of flies who sensed the banana and traveled up to it in 30 seconds. Bananas were chosen as it is a popular household

item that fruit flies are attracted because of their sugar content. The 30 second time interval was chosen as it provided ample time for the flies to detect the food source to most accurately reflect the strength of their olfactory receptors.

IV. Results and Discussion

In this experiment, flies that were not exposed to vapor were compared against flies exposed to secondhand vapor and thirdhand vapor separately. This represented people with no exposure to e-cigarette vapor and people in contact with vapor in its gaseous form and residual state, respectively. The following results convey the varying degrees of severity that e-cigarettes pose to youth and adult populations in terms of their geotaxis and olfactory perceptions.

Secondhand Vapor Exposure:
Secondhand e-cigarette vapor includes both vapor emitted from the vape device and vapor breathed out by the person vaping the e-cigarette [15]. This study mainly focused on vapor emitted from the vape device, as vapor breathed out from the smoker was not produced. Exposure to this type of vapor has previously been known to cause respiratory diseases such as lung cancer, in addition to causing damage to the heart as many chemicals in e-cigarette vapor are known carcinogens or irritants [15].

Exposure to secondhand e-cigarette smoke caused the geotaxis of *Drosophila melanogaster* to increase as significantly lower heights were recorded after exposure. Furthermore, larvae fruit flies exhibited a slightly greater decrease in height than exposed adults. Flight heights of the larvae decreased by 52.8%, while adult flight heights decreased by 47.9%. This deviation from normal behavior is shown in Figure 1, which summarizes the geotaxis data for flies exposed to secondhand vapor. It can also be seen from the data that heights for both larvae and adults only decreased substantially after at least four days of exposure, showing that exposure for short periods of time may not have drastic effects on movement perception. For instance, someone who is in the vicinity of a smoker only one time will likely not experience clear negative effects.

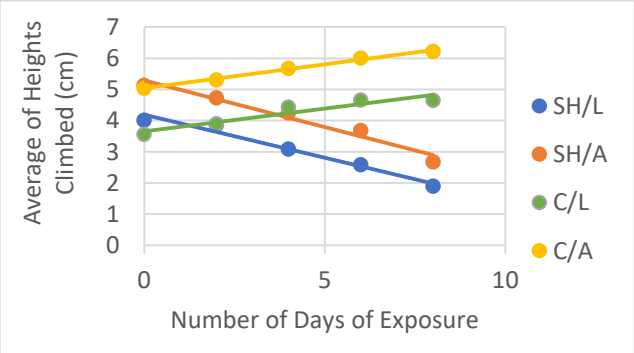

Figure 1: Geotaxis data comparing flies exposed to secondhand e-cigarette vapor with flies in the control group not exposed to vapor. The heights of flies after zero and eight days of exposure are shown. Secondhand exposure is written as "SH", the control group is written as "C", and Larval and Adult populations are denoted by "L" and "A", respectively.

Fruit flies exposed to secondhand vapor demonstrated a weakened ability for sensory perception. The percentage of flies who were able to sense the fruit significantly decreased, as shown in Figure 2. Larvae able to sense the fruit decreased by more than 50% after eight days of exposure. Adults that were exposed decreased by approximately 40%. However, flies not exposed to vapor displayed a greater ability to sense fruit as larvae displayed a 12% increase in olfactory ability while adults displayed a 4% increase. This increase may be attributed to increased acclimation to the environment, or the possibility of food sources that may have been located in the surroundings. Overall, when comparing flies that had not been exposed, sensory perception of *Drosophila melanogaster* proved to be diminished due to vapor exposure.

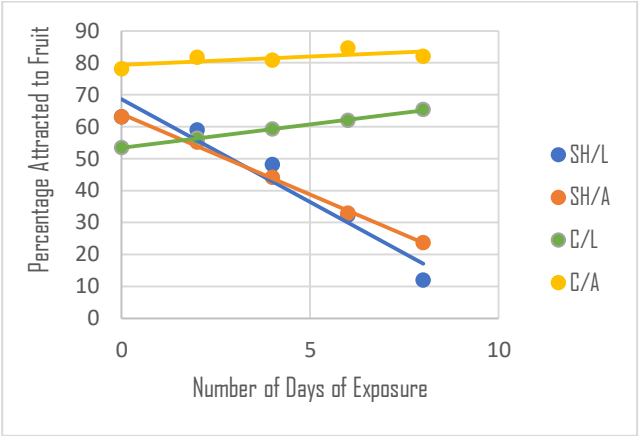

Figure 2: Olfaction data comparing flies exposed to secondhand e-cigarette vapor with flies in the control group not exposed to smoke. The percentage of flies that sensed the fruit after zero and eight days of exposure is shown. Secondhand exposure is written as "SH", the control group is written as "C", and Larval and Adult populations are denoted by "L" and "A", respectively.

Thirdhand Vapor Exposure:
Thirdhand vapor exposure includes residue from e-cigarettes that collects on various surfaces including walls, windows, and clothing [16]. Exposure to this residue through touch, ingestion, or inhalation are shown to damage DNA and cause diseases such as asthma and cancer [16].

Drosophila melanogaster flew at lesser heights after being subject to thirdhand e-cigarette vapor exposure. Heights for larvae decreased by approximately 62%, while heights for adults decreased by only 48%. It can be predicted that the difference between larval and adult movement perception will increase as exposure is enacted more frequently and for a longer period of time. The control group that was not exposed to vapor exhibited a slight increase in height, with larval perception increasing by 31% and adult perception increasing by 19%, proving that e-cigarette vapor residue has toxic effects on the movement of fruit flies.

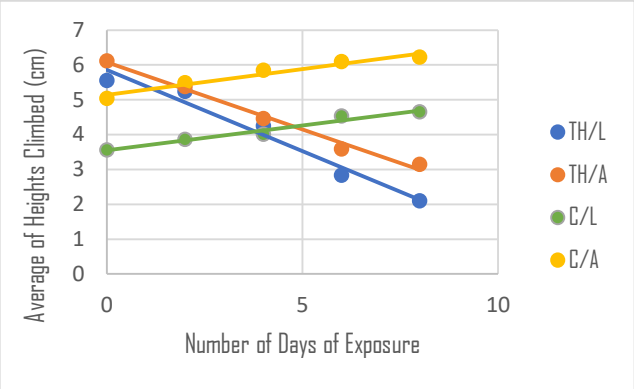

Figure 3: Geotaxis data comparing flies exposed to thirdhand e-cigarette vapor with flies in the control group not exposed to smoke at zero and eight days of exposure. Thirdhand exposure is written as "TH", the control group is written as "C", and Larval and Adult populations are denoted by "L" and "A", respectively.

Residue left on sponges contributed to a negative effect on the olfaction strength of *Drosophila melanogaster*. Both larvae and adults experienced similar effects on olfactory strength after exposure, with 37% less larvae and 42% less adults able to detect the food. Comparatively, olfaction rates for flies not exposed to smoke increased by 4-15% for larvae and adults. Although it can be said that olfaction is negatively affected by thirdhand e-cigarette vapor exposure, it is unclear if larval populations are affected more severely than adult populations. Further study concerning prolonged exposure may resolve this obscurity.

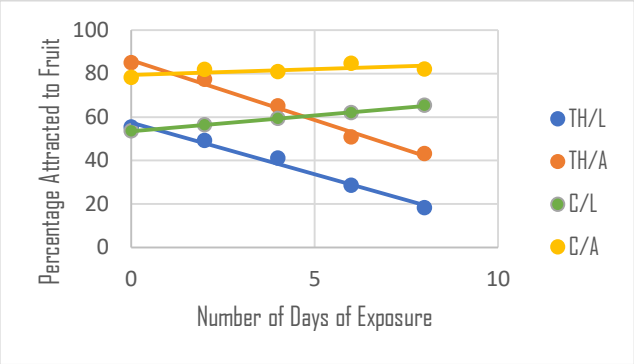

Figure 4: Olfaction data comparing flies exposed to thirdhand e-cigarette vapor with flies in the control group not exposed to smoke at zero and eight days of exposure. Thirdhand exposure is written as "TH", the control group is written as "C", and Larval and Adult populations are denoted by "L" and "A", respectively.

Data averaged across all five trials for each type of exposure is shown in the table below.

Table 1: Data averaged across all five trials for secondhand vapor exposure

	Secondhand Exposure			
	Larvae		*Adult*	
	Geotaxis (cm)	*Olfaction (% of flies)*	*Geotaxis*	*Olfaction*
0	4.01	63.02	5.13	63.03
2	3.86	58.96	4.72	55.18
4	3.08	48.16	4.25	44.16
6	2.58	32.28	3.68	32.88
8	1.90	11.96	2.67	23.67

Table 2: Data averaged across all five trials for thirdhand vapor exposure

	Thirdhand Exposure			
	Larvae		*Adult*	
	Geotaxis (cm)	*Olfaction (% of flies)*	*Geotaxis*	*Olfaction*
0	5.55	55.36	6.11	84.80
2	5.24	49.12	5.37	77.22
4	4.25	41.06	4.46	64.96
6	2.83	28.52	3.59	50.68
8	2.10	18.18	3.15	43.12

Table 3: Data averaged across all five trials for control groups for comparison

	Larvae		**Adult**	
	Geotaxis (cm)	*Olfaction (% of flies)*	*Geotaxis*	*Olfaction*
0	3.56	53.48	5.04	78.08
2	3.9	56.34	5.31	81.74
4	4.43	59.26	5.68	80.76
6	4.65	61.92	6.00	84.64
8	4.65	65.35	6.22	81.98

Overall, the results of this experiment reveal that both secondhand and thirdhand exposure to e-cigarette vapor do in fact negatively affect the geotaxis and olfactory perceptions of *Drosophila melanogaster*.

V. Conclusion and Future Scope

In this study, the effects of secondhand and thirdhand e-cigarette vapor exposure were examined by analyzing the geotaxis and olfaction of larval and adult *Drosophila melanogaster*. It was proved that e-cigarette exposure is harmful to the human body, but also that youth aged 12-17 may be subject to more harmful effects than adults, including but not limited to neurological effects.

The experiments showed that the geotaxis of flies exposed to secondhand and thirdhand vapor was negatively affected, as shown by significantly lesser heights flown by flies after eight days of exposure. In addition, for both secondhand and thirdhand vapor exposure, the olfactory strength of the flies also decreased, shown by lesser percentages of flies being attracted to fruit placed at the top of the environment after successive exposures. In both these tests, flies that had been exposed to vapor as larvae performed more poorly on the tests than flies who had only been exposed as adults.

All of these results provide important insight into the effects of e-cigarette vapor on *Drosophila melanogaster* that may be translatable to the human body. Specifically, vapor exposure was found to cause dulled movement and sensory perception in the brain. Further experimentation may focus on the effects of prolonged exposure over a full lifetime to provide potential long-term health expectations for e-cigarette users, as the current experimental procedures only included exposure for approximately half of the flies' lifespan. This experiment may help combat the epidemic of new vaping related cases and establish further regulations concerning the chemicals used in the manufacturing and preparation of these devices.

REFERENCES

[1] Cullen KA, Ambrose BK, Gentzke AS, Apelberg BJ, Jamal A, King BA. Notes from the Field: Increase in use of electronic cigarettes and any tobacco product among middle and high school students – United States, 2011-2018. MMWR Morbidity & Mortality Weekly Report 2018; Vol. 67, No. 45, pp.1276-1277, 2018.

[2] Office of the Surgeon General. E-cigarette Use among Youth and Young Adults: A Report of the Surgeon General Cdc Washington, DC: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2016.

[3] Office of the Surgeon General. The Health Consequences of Smoking-50 Years of Progress: A Report of the Surgeon General Cdc, Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention (US), National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014.

[4] Hi Kalpana. I. Pattan and Sridevi I. Puranik, "*Occupational Health Hazards of Women Beedi Rollers in Nippani Taluk, South India*", International Journal of Scientific Research in Biological Sciences, Vol.6, Issue.3, pp.130-132, 2019

[5] Lung Injury Response Laboratory Working Group. Vitamin E Acetate in Bronchoalveolar-Lavage Fluid Associated with EVALI. United States, 2020

[6] Diez-Izquierdo A, Cassanello-Penarroya P, Lidon-Moyano C, Matilla-Santander N, Balaguer A, Martinez-Sanchez JM. Update on thirdhand smoke: A comprehensive systematic review. Environ Res. 2018; Vol. 167, pp. 341-371, 2018.

[7] Jyostna.V, Anuhya.G, Ushakiranmayi. Managamuri, Srinuvasulu K, Sudhakar. Poda, "*Outcome of Cadmium on Aminergic System and Protective Function of Vitamin-C*", International Journal of Scientific Research in Biological Sciences, Vol.5, Issue.2, pp.18-23, 2018

[8] Hang B, Sarker AH, Havel C, et al. Thirdhand smoke causes DNA damage in human cells. Mutagenesis. 2013; Vol. 28, Issue. 4, pp. 381-91.

[9] Watkins SL, Glantz SA, Chaffee BW. Association of Noncigarette Tobacco Product Use With Future Cigarette Smoking Among Youth in the Population Assessment of Tobacco and Health (PATH) Study, 2013-2015. *JAMA Pediatr*. Vol. 172, No. 2, pp. 181–187, 2018.

[10] Julia Cen Chen-Sankey, Jennifer B. Unger, Maansi Bansal-Travers, Jeff Niederdeppe, Edward Bernat, Kelvin Choi; E-cigarette Marketing Exposure and Subsequent Experimentation Among Youth and Young Adults. *Pediatrics* November 2019; Vol. 144 No. 5, 2019

[11] Wagoner KG, Reboussin DM, King JL, Orlan E, Cornacchione Ross J, Sutfin EL. Who Is Exposed to E-Cigarette Advertising and Where? Differences between Adolescents, Young Adults and Older Adults. *International Journal of Environmental Research and Public Health*. Vol. 16, No. 14, pp. 25-33, 2019.

[12] Michael Siegel, Amanda Katchmar, Effect of flavored E-cigarette bans in the United States: What does the evidence show?, Preventive Medicine, 107063, ISSN 0091-7435, 2022.

[13] Hi Linderman JA, Chambers MC, Gupta AS, Schneider DS (2012) Infection-Related Declines in Chill Coma Recovery and Negative Geotaxis in Drosophila melanogaster. PLoS ONE Vol. 7 Issue. 9, e41907

[14] Vosshall, Leslie B, Olfaction in Drosophila, Current Opinion in Neurobiology, Volume 10, Issue 4, pp.498-503, 2000. ISSN 0959-4388.

[15] Wang TW, Marynak KL, Agaku IT, King BA. Secondhand exposure to electronic cigarette aerosol among U.S. youths. JAMA Pediatr, Vol. 171 Issue. 5, pp. 490, 2017.

[16] Hi Thomas JL, Hecht SS, Luo X, Ming X, Ahluwalia JS, Carmella SG. Thirdhand tobacco smoke: a tobacco-specific lung carcinogen on surfaces in smokers' homes. Nicotine Tob Res., Vol. 16 No. 1, pp. 26-32, 2013.

AUTHORS PROFILE

Srilekha Mamidala is a high school student at Garnet Valley High School in Glen Mills, Pennsylvania. Srilekha has performed research in various fields, including computational biology, computer science, and environmental toxicology, among others. She is especially interested in finding creative computational solutions to biological issues.

