The Journal of Biological Sciences

The Developmental effects of Formaldehyde on *Drosophila melanogaster*

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Edited by Daniel Pinolini

Abstract

Formaldehyde is a common volatile organic compound (VOC) found in the environment that is known to cause detrimental health effects in humans and some animals. Higher levels of formaldehyde can be found in indoor settings due its popularity in industry and household goods, especially in the forms of air fresheners. It is a common misconception that air fresheners remove odorous compounds in the air, however they do the opposite by adding pleasant smelling compounds in the air to mask the scent of odorous ones. There has been previous work that explored the behavioral and genetic effects that formaldehyde may cause in Drosophila melanogaster; however, few have been done with regards to their development. This study investigated the effects of formaldehyde on the development *of Drosophila melanogaster* by measuring egg hatching percentage. The results indicate that formaldehyde does not have a significant effect on egg hatching.

Introduction

Air fresheners are common household goods that can be found in any indoor settings, and they come in many forms: scented candles, diffusers, gels, and sprays. This makes it easy to introduce air fresheners into indoor settings. It is a common misconception that air fresheners reduce the amount of odors in the air. This is not true, however, as air fresheners add more chemicals to the air to mask the bad odors with pleasant-smelling fragrances, rather than removing them (Uhde and Schulz, 2015). Air fresheners have been found to contain harmful, volatile organic compounds (VOCs) that can cause detrimental health effects in humans (Kim et al., 2015). VOCs are hydrocarbon compounds with low boiling points, which allows them to evaporate easily in the air thus creating a high vapor pressure. These compounds can also generate ozone and other odors in the air (Kim et al., 2015). Common VOCs that can be found in air fresheners are formaldehyde and toluene, both which have been found to cause detrimental health effects in humans and animals (Kim et al., 2015). On average, air fresheners contain 51 $\mu g/m^3$ to 69 $\mu g/m^3$ of formaldehyde and 0.4mg/kg to 11.9/kg of toluene (Kim et al., 2015). Long term exposure to these amounts of formaldehyde and toluene can damage human health. Some potential negative health effects that formaldehyde can cause in organisms are eye, nose, and throat irritation, dizziness, diarrhea, and impairs memory functions. Exposure to high concentrations of formaldehyde for an extended period of time can cause eye irritation, vomiting, spasms and death. Even exposure to small amounts of formaldehyde can cause sensory irritation (Kim et al., 2015). Air freshener associated-symptoms can be difficult to identify because deleterious effects may not manifest for many years (Kim et al., 2015). Pregnant women and their developing fetus in particular are more vulnerable to harmful components during their pregnancy term (Xu et al., 2017). This makes it even more important for people to limit their exposure to formaldehyde as much as they can since humans and some animals spend most of their lives indoors.

Formaldehyde is a colorless, transparent gas that is volatile at room temperature, making it difficult to identify the presence of formaldehyde in the air. Due to this, it can be hard for people to know exactly how much formaldehyde is in their homes. In addition, it is very easy to introduce formaldehyde into an indoor setting because many people are unaware of the industrial utilization of formaldehyde. Formaldehyde can be found in many wood-based materials and furniture, paper products, paints, cleaning materials and cosmetics (Salthammer et al., 2010). Formaldehyde has been classified as a human carcinogen by the International Agency for Research on Cancer (IARC) in 2004 and by European Commission (EU) in 2014. The EU classified formaldehyde as a 1B carcinogen, which indicates that carcinogenic effects have been observed in animal trials and are probable for humans (Salthammer, 2015).

D. melanogaster is an attractive model organism for studying human health due to the many pharmacological and physiological similarities that they share with humans. *D. melanogaster* are considered to have the greatest similarities with humans amongst non-mammalian animals (Abnoos et al., 2013). In addition to

these similarities, *D. melanogaster* are inexpensive and can be easily cultivated and maintained in a lab setting, thus making them the perfect model organism for this experiment. The advantage of using *D. melanogaster* in studying development and reproduction is that they have a short life cycle and can generate many generations with a large amount of progeny in a short time frame (Hales et al., 2015). Each female *D. melanogaster* can lay up to ~100 eggs a day for up to 20 days (Jennings, 2011). When kept under 25^{0}C, it takes approximately 7 days for *D. melanogaster* to complete a full life cycle from egg to fertile adult flies (Hales et al., 2015). *D. melanogaster* can also be commonly found in any indoor settings where they can be easily exposed to the formaldehyde in air fresheners. There have been numerous studies concerning the behaviors and genetic alterations that occur within *D. melanogaster* when exposed to different substances. There are, however, only a very limited number of studies that focuses on the changes in reproduction and the lifespan of *D. melanogaster* that may occur upon exposure to formaldehyde (Miah et al., 2013).

The purpose of this study is to explore the effects that formaldehyde may have on the reproductive success of *D. melanogaster*. It was predicted that eggs exposed to formaldehyde would have a lower average hatching percentage. To determine this, the *D. melanogaster* eggs were exposed to different concentrations of formaldehyde and a formaldehyde-free natural air freshener during their embryo stage of development. One group of flies received no treatment at all, which establishes a true control for the experiment where the flies' natural development can be observed. The exposure to a formaldehyde-free natural air freshener is to compare how the absence of formaldehyde in air fresheners may affect the hatching percentage of fruit flies. The average hatching percentage for each trial was recorded after 24hrs of treatment.

Materials & Methods

Organism Maintenance

Two vials containing wild type *D. melanogaster* (~10-12 flies per a vial) were obtained from Carolina Biological Supply and were kept in an incubator at 23^{0}C. The flies were transferred into new vials every 3-4 days in order to prevent overcrowding as they reproduce. The new vials were filled one third with instant fly medium obtained from Carolina Biological Supply and distilled water. The medium was gently patted down to the bottom of the vial and a twisted napkin was placed in the vial to increase surface area and prevent flies from getting stuck in the medium. Flies were exposed to small amounts of CO_2 gas in order induce temporary paralysis for easy transfer between vials.

Collection of Eggs and Embryos

Ten female and five male fruit flies were kept in embryo collection chambers for mating and were placed in the incubator. Eggs were collected daily from fresh oviposition plates made from agar (10g), sucrose (10g), p-hydroxybenzoic acid (0.6g), 300mL distilled water and 100mL Welch's grape juice. Prior to egg laying, the center of the agar was sprinkled with 20mg of active yeast in order to induce eggs laying in female flies (Becher et al., 2012). Eggs were transferred to new agar plates using a tungsten needle within 24hrs of being laid. Exactly 20 eggs were placed on each new agar plate.

Treatment

In the new agar plates, each egg was treated with 0.5µL of 50ppm formaldehyde, 100ppm formaldehyde or a formaldehyde-free natural air freshener (*Natural Flower Power*). In order to establish a true control, the eggs in one plate received no treatment at all. The plates were kept in the 23^{0}C incubator and the hatching percentage for each treatment was recorded after 24hrs of treatment.

Statistics

The hatching percentage were presented as mean (of 4 replications) ±SEM. One-way ANOVA was calculated using Microsoft Excel 2016. Graph were also plotted using the same software.

Results

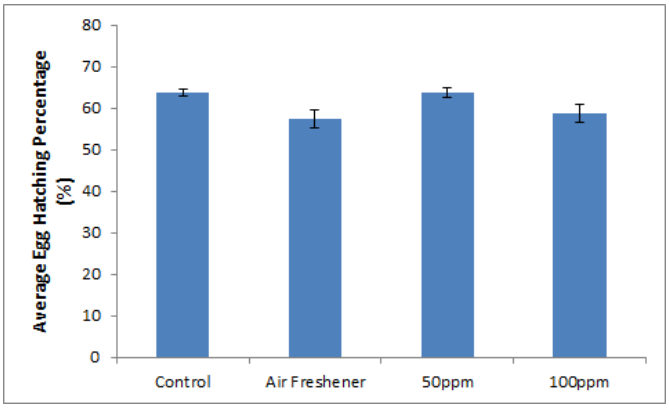

Figure 1. The average hatching percentage of eggs after treatment: nothing (control), formaldehyde-free natural air freshener (*Natural Flower Power*), 50ppm formaldehyde and 100ppm formaldehyde.

To test our hypothesis, we measured the rate of egg hatching with different amount of chemical exposures (Materials and Methods). The average hatching percentage of eggs for all treatment groups were similar. The eggs in the control and the eggs treated with the 50ppm formaldehyde had the highest hatching

percentage of all treatments, while the eggs treated with the formaldehyde-free natural air freshener yielded the lowest hatching percentage (Fig. 1). Therefore, we concluded there were no significant differences in the hatching percentage between our control and treatment groups (Fig. 1, $p = 0.92$).

Discussion

Formaldehyde had no adverse effects on the hatching percentage of *D. melanogaster* eggs in our current experimental conditions. This may be because the concentrations of formaldehyde used in the experiment were too small to have an effect on the egg hatching percentage. It may also be possible that the treatments affected one another in the incubator due to the volatility of the material used and the inability to cover the mesh on top of the cages. The treated eggs maintained a steady survival rate from egg to larvae that was relatively high. However, the survival rate from larvae to pupae and from pupae to adult were not observed due to the experimental design and time constraints. Therefore, our current experiment could not address if formaldehyde had any long-lasting effects on the health of the flies.

The high survival rate from egg to larvae may be due to the antimicrobial properties of formaldehyde. Formaldehyde has antimicrobial properties against bacteria at levels as low as 1 ppt (Guerfali et al., 2011). This can help increase the hatching percentage of *D. melanogaster* eggs by reducing the amount of microorganisms on the eggshells, making it easier for the larvae to hatch. The eggshells of *D. melanogaster* carry low amounts of microorganisms (bacteria) of high diversity (Broderick and Lemaitre, 2012), therefore formaldehyde exposure may further decrease the amount of bacteria on the surfaces of the eggs. As a result, when the larvae consume the eggshell, it is consuming less bacteria that may be beneficial to their guts as adults. Gut bacteria plays an important role in the development of *D. melanogaster*. Gut bacteria are not inherited from parent to offspring but are acquired from the environment after birth (Broderick and Lemaitre, 2012). The first instar larvae consume their eggshells after hatching and the microorganism on the eggshells becomes a part of their gut bacteria. The bacteria density in the gut increases throughout the larval stages of development until pupation. During the transition period from larvae to pupa, there is a sharp decrease in bacteria density, which is then restored 48 hours after pupation (Broderick and Lemaitre, 2012). Gut-associated bacteria also plays an important role in the longevity *of D. melanogaster*. Flies raised under axenic conditions or treated with antibiotics experienced a shorter lifespan than the flies that were raised under normal conditions (Brummel et al., 2004).

Only the egg hatching percentage was observed in this experiment and no data was collected on how many of larvae survived to adulthood or the effects that the treatment had on the adult's ability to reproduce. Negative developmental effects of formaldehyde however, have been observed in the development of *Ceratitis capitate* (Mediterranean fruit fly) after treatment (Guerfali et al., 2011). The hatching percentage in the eggs treated with 100ppm formaldehyde increased by ~20% compared to the control. In addition, adult males in the treatment groups experienced infertility issues. It is unknown, however, whether this would be observed in *D. melanogaster* eggs because *C. capitate* and *D. melanogaster* belong to different families despite being in the order (Diptera). Further experimentation would be necessary to determine whether formaldehyde has long lasting negative impacts on developmental success in *D. melanogaster*.

Since the development process beyond hatching could not be observed, future studies can be performed in order to observe whether formaldehyde exposure has an effect on the adult flies and their progeny. By performing a generational study, the effects of formaldehyde on both egg hatching and the development process can be observed in different generations in the same lineage of flies. This also provides insights on the long-term effects of formaldehyde in different generations of fruit flies after exposure.

Acknowledgements

We would like to thank Dr. Lee for this research opportunity, Daniel Pinolini for assisting us throughout this experiment and Cody Stevens for guiding and assisting us throughout the whole learning process with working with the flies.

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