Summer 8-15-2021

Using circadian rhythm disruption as a readout of the effects of lead toxicity on behavior

Sarah John '24
Hamilton College

Dylan Koproski '23
Hamilton College

Using circadian rhythm disruption as a readout of the effects of lead toxicity on behavior

Sarah John, Dylan Koproski, Professor Rhea Datta

Abstract

Prior literature has indicated that toxic metal exposure can alter the circadian clock rhythm and lead to biological and physiological dysfunction. The purpose of this research was to study the impact of chronic exposure to lead on the circadian rhythm patterns of *Drosophila Melanogaster*. Fly locomotive activity was used as a measure of strength of the circadian rhythm after exposure to lead. It was hypothesized that higher concentrations of lead would have a greater impact on the circadian locomotor activity patterns of exposed *Drosophila*, specifically on the transition periods between "Morning" and "Evening." Young adult male flies(1-3 days post-eclosion) were exposed to concentrations of 100 μg/ml and 75 μg/m of lead for their entire development from egg to eclosion. This experiment uses the Drosophila Activity Monitor (DAM) System to record fly locomotor activity over six 24-hour cycles. The results produced a statistically non-significant but positive correlation between lead concentration and change in locomotor activity at transition periods from "Morning" to "Evening."

1. Introduction

Lead (Pb) is a highly toxic, naturally occurring heavy metal that has been observed to have detrimental impacts on humans and the environment. This toxin has been widely used for several centuries because of its malleability, resistance to corrosion, and low melting point. Due to its long history of global use, lead is ubiquitous in the environment with its most common contributors being leaded gasoline, paints, ceramics, ammunition, water pipes, and personal care products (Wani et al., 2015). Although regulatory interventions have resulted in significant reductions in Pb use, lead exposure to human populations still persists. This has created a major

public health issue that has been responsible for countless developmental abnormalities annually. Today, the general population may be exposed to Pb in air, foods, drinking water, soil, dust and other sources (Frank et al., 2019). Since it does not degrade, Pb has the potential to bioaccumulate and biomagnify over time. In humans, studies have indicated that Pb can have negative impacts on almost every organ system in the human body (Jaishankar et al., 2014). On a biological level, Pb can cause oxidative stress and lipid peroxidation in the liver, kidney, brain, and red blood cells (Aykin-Burns et al., 2002). As such, it is crucial to further understand the impacts of lead on the human body in order to produce adequate treatments for those affected. The present study was designed to analyze the impact of exposure to lead on the circadian rhythm patterns of *drosophila melanogaster*.

Circadian rhythms are behavioral, molecular, and physiological changes that occur in response to a roughly 24 hour cycle of lightness and darkness. These rhythms are driven by internal molecular clocks that allow an organism to anticipate rhythmic changes in their environment. In most mammals, the circadian clock orchestrates rhythms of sleep-awakeness, body temperature, blood pressure, circulating hormones, metabolism, among other biological events. Circadian rhythms are defined by four properties: They (1) repeat once a day over a 24 hour period, (2) are endogenous and persist in the absence of external cues, (3) can be reset by normal changes in environmental conditions, and (4) the rhythms remain stable over a wide range of temperatures (Tartaroglu & Emery, 2014). Prior literature indicates that long term, harmful stimuli can alter the clock rhythm and lead to biological and physiological dysfunction. For example, factors such as genetic mutations, long-term sleep disruption due to jet lag, shift-work, or artificial lighting, and toxins can result in sleep disorders, cardio/metabolic disorders, and behavioral/mental disorders. In particular, toxic metal exposure, including lead,

has been implicated in the disruption of circadian rhythms and the acceleration of neurodegenerative disorders, such as Parkinson's and Alzheimer's disease (Tartaroglu & Emery, 2014).

Circadian rhythms are perhaps best understood in the model organism *Drosophila melanogaster*, more commonly referred to as fruit flies. In fact, it was in *Drosophila* where the clock mechanism was first discovered. In *Drosophila*, the circadian transcription factors CLOCK (CLK) and CYCLE (CYC) promote the transcription of the *period(per) and timeless (tim)* genes in a negative feedback loop (Vitaterna et al, 2001). The fly clock is highly conserved with the human clock, with *Drosophila* having direct homologs with mammalian circadian transcription factors. In addition, the *Drosophila* genome is 60% homologous to that of humans, with 75% of genes involved with human diseases having homologs in flies (Mirzoyan et al., 2019). Thus, the similarities between *Drosophila* and humans, coupled with their short lifespans and small size, make the fruit fly an attractive model for large-scale circadian rhythm studies, the results of which may also be generalized to humans.

The *Drosophila* circadian rhythm can be best assessed by measuring fly locomotive activity. Most studies utilize the *Drosophila* Activity Monitoring (DAM) system (TriKinetics) to generate an actogram that measures fly activity over several day/night cycles (Image A). In typical conditions of light-dark (LD), where lights alternate between 12 hours light and 12 hours dark, flies show a morning activity peak (M) in lights-on and an evening peak (E) in lights-off. In addition, flies display substantial bursts of locomotor activity during the transitions of lights-on and lights-off, known as the "masking transition period" (Tartaroglu & Emery, 2014). Prior literature indicates that flies monitored in constant darkness (DD) also display activity bouts on a 24 hour cycle, similar to that of the LD condition. Therefore, any deviations in the *Drosophila*

circadian rhythms after exposure to lead is significant as it may be indicative of disruption to the fly's internal processes.

The overall of the following study was to determine the toxicological impact of lead on *Drosophila Melanogaster* circadian rhythms by measuring locomotive activity. This study used young, male, wild-type fruit flies (1-3 days post eclosion) grown on a constant day-night cycle. In the lead stocks, parent F0 flies laid eggs in lead-dosed food tubes, of which their offspring, the F1 generation, ate throughout their development from larvae to adulthood. In this experiment, the following concentrations of Pb were used: Control (0 µg/mL), 100 µg/mL, and 75 µg/mL. Due to time constraints, data from the control and 100 µg/mL flies were collected and analyzed, while only preliminary data was collected for the 75 µg/mL flies. It was hypothesized that higher concentrations of lead would have a greater impact on the circadian locomotor activity patterns of affected *drosophila*, specifically on the masking transition periods, the bursts of activity between the transition of day and night. This study produced a statistically non-significant but slight positive correlation between lead concentrations and change in locomotor activity at the masking transition periods.

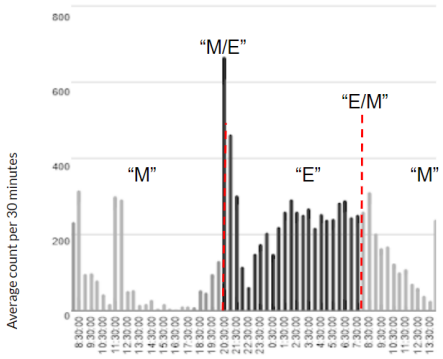

Image A: Sample normal circadian rhythm activity in *Drosophila Melanogaster.* "M" stands for "Morning" or lights on and "E" for "Evening" or lights off.

2. Methods:

2.1 Food Preparation:

Nutri-Fly® Premixed Drosophila Media powder from Genesee Scientific was used to produce all the fly food for this experiment. To make the food, a packet of the media powder was mixed vigorously with deionized water in an Instant Pot. The mixture was then left to pressure cook on the "sealing" setting and then left to depressurize before opening. Throughout the procedure, the pot was set to 70℉ to ensure that the food mixture did not harden. Next, 10% Tegosept and Propionic Acid were incorporated into the mixture to ensure maximum shelf life. For the lead food concentrations of 100 and 75 μg/mL, calculated amounts of 1000 μg/mL of lead were added to the food mixture to produce the desired concentrations. No lead was added to the control food.

Next, 10mL of each food was pipetted into vials labeled with their respective concentrations. A cheesecloth was placed over the vials of food, which were left to harden in a cool, dry place overnight. The food was then capped with cotton and any unused food was placed in the fridge to prevent spoilage.

2.2 Drosophila preparation:

For consistency, flies were grown in an incubator set on a day/night cycle with lights that switched on at 08:00 every morning and turned off 12 hours later at 20:00 at night. Wild type flies were flipped into tubes with food of the desired lead concentration and left for 1-3 days in order to lay eggs. The parent flies were then dumped and their offspring were left to grow in the incubator. Approximately 1-3 days after the pupae enclosed, the flies were knocked out with CO_2 gas and separated based by sex. The females were returned back to the vial, while the males were prepared for the activity monitor.

2.3 Activity monitor setup:

In this experiment, two 4-beam DAM5M monitors(TriKinetics) were connected to a dedicated computer in a dark room. Both monitors were then placed inside another incubator set on a 24 hour day-night cycle, with lights turning on at 8 a.m. and lights turning off at 8 p.m. On the computer, two software programs were installed from TriKinetics: DAMSystem311 and USB Drivers 1808. The PSIU9 power supply unit from the activity monitors was then connected to a wall outlet and then to the computer. The DAMSystem311 software was then set to record data at an interval of 30 minutes for both activity monitors.

2.4 Data collection and Analysis:

The DAM assay passes an infrared beam, which has no effect on the circadian clock, through an individual small glass tube holding a singular fly over several days. The DAM monitor is then placed in an incubator with a day/night cycle and the number of times the fly passes through the beam is recorded.

In the experiment, control food and a rubber stopper was inserted into one end of 5mm glass pyrex tubes. A sleeping male fly, previously knocked out with CO_2, was then inserted into each tube using a thin paintbrush. Lastly, cotton was placed onto the other non-food end of the tube. The tubes were then placed horizontally in the activity monitor in the incubator and left undisturbed for 7 full days (6 day-night cycles) (Image B). At the end of the experiment, the data was then collected from the DAM system and formatted to create actograms in google sheets.

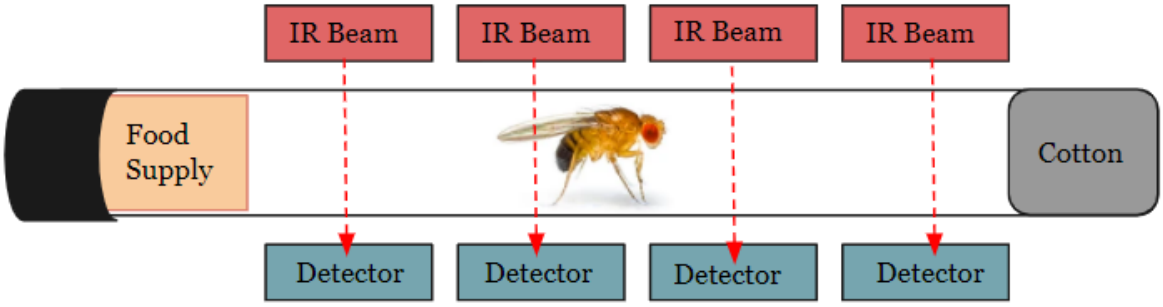

Image B: Illustration of a DAM tube containing a fly.

3. **Results:**

For our control experiments, the flies exposed to 12 hours of light and 12 hours of darkness exhibited a clear diurnal rhythm pattern with more activity during the night than during the day. In addition, at the masing transition periods between the light switches, noticeable spikes of activity were present every cycle (Fig. 1). The amplitude of the peaks of the masking transition periods, specifically between the transition from “Morning” to “Evening” appeared to peak on the second cycle before steadily decreasing with time.

Male flies exposed to 100µg/mL of lead experienced similar locomotive activity patterns to that of the control flies. The lead flies had significantly more activity at night than during the day, as well as a gradual decrease in the amplitude of the masking transition peaks.

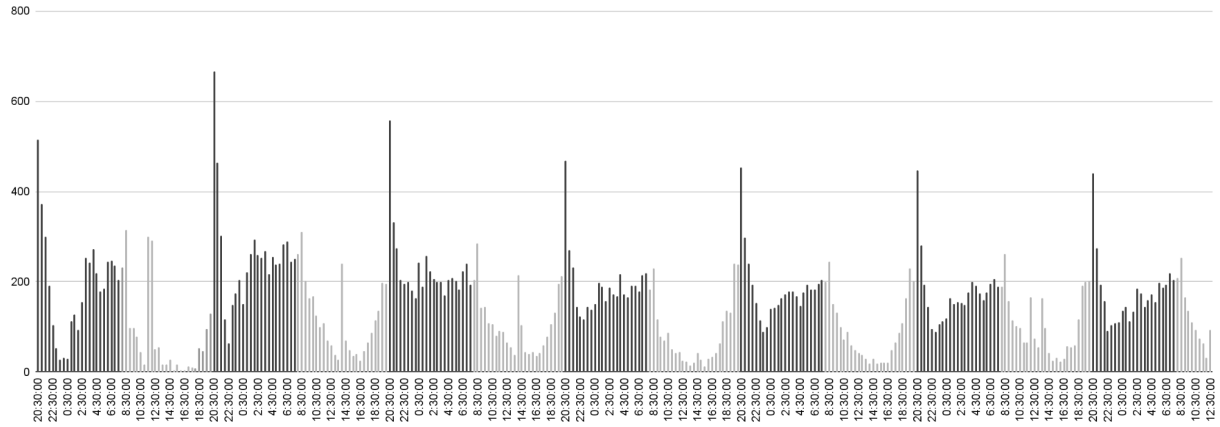

Fig. 1: Actogram of the average activity of 20 control flies over the span of a week. Light grey denotes lights on and dark grey denotes lights off.

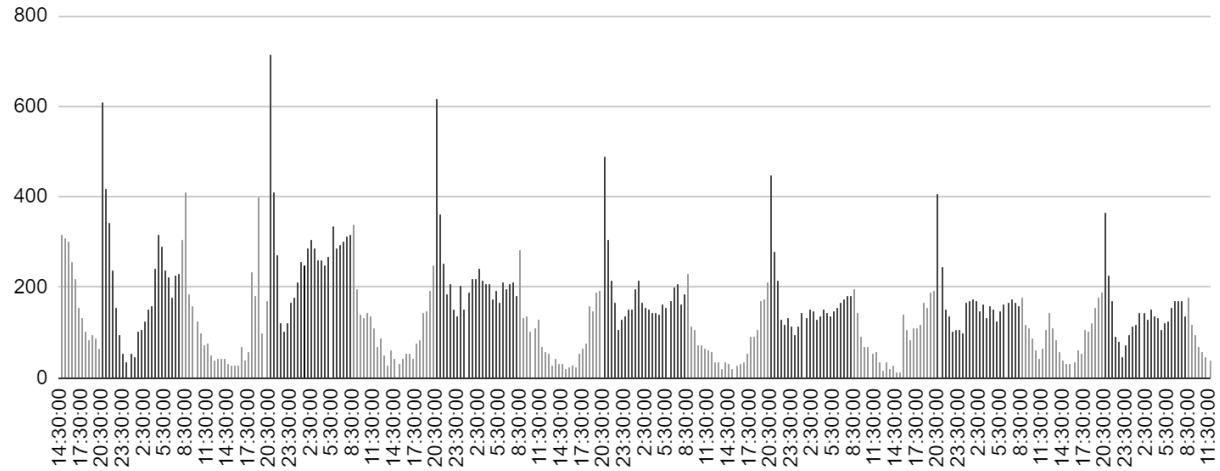

Fig. 2: Actogram of the average activity of 20 lead flies dosed with 100µg/mL over the span of a week. Light grey denotes lights on and dark grey denotes lights off.

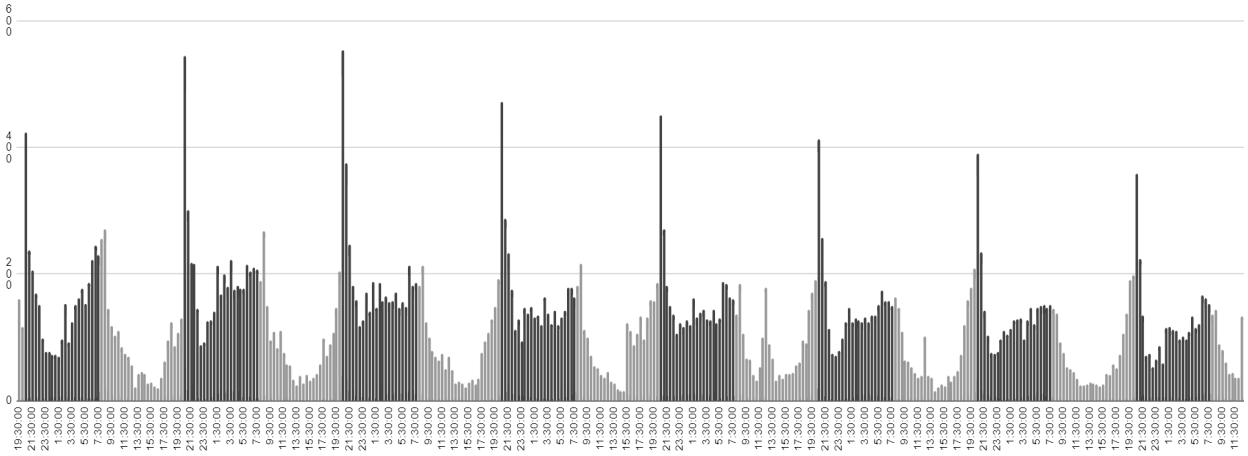

Fig. 3: Actogram of the average activity of 20 lead flies dosed with 100µg/mL over the span of a week. Light grey denotes lights on and dark grey denotes lights off.

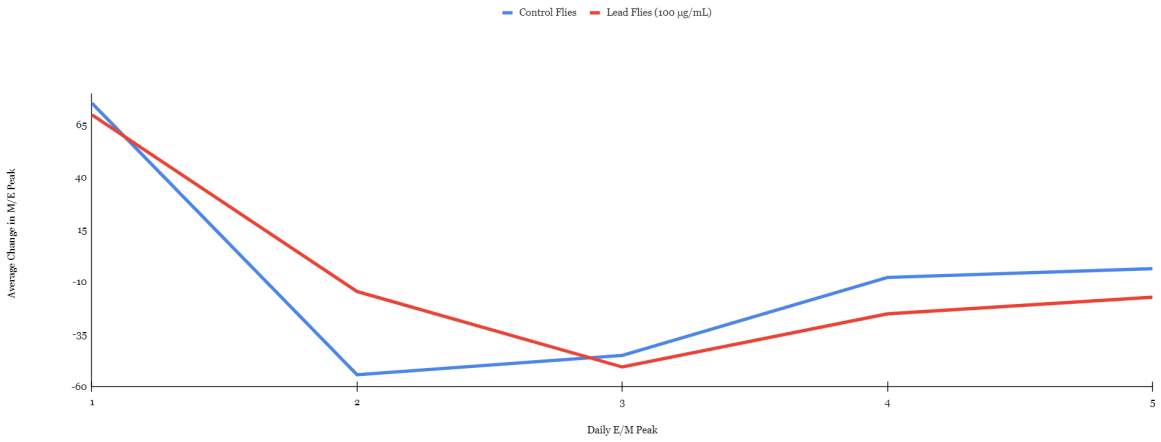

Fig. 4: Average rate of changes of E/M peaks over time comparing 100µg/mL lead group and control. $p = 0.170835$

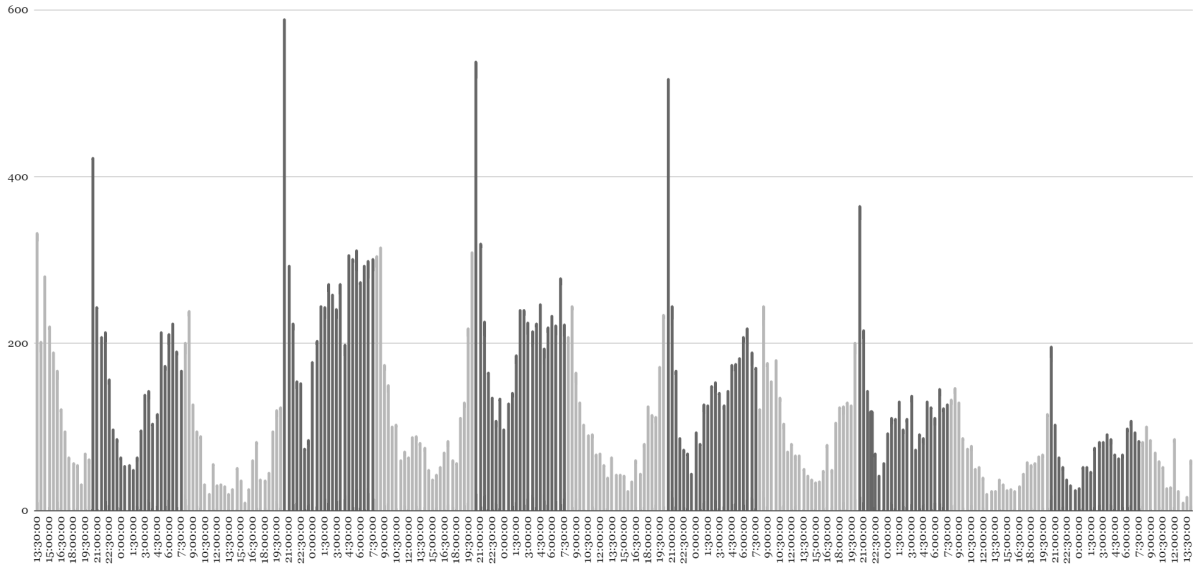

Fig. 5: Actogram of the average activity of 20 lead flies dosed with 75µg/mL over the span of a week. Light grey denotes lights on and dark grey denotes lights off.

4. Discussion

The findings of our control studies support previous research showing that male, young wild type flies have rhythmic locomotive activity patterns over the course of 24 hour circadian days. In the experiment, control flies exhibited bouts of morning and evening activity, as well as a distinct peak between the transition from morning to evening (Fig.1). The flies showed more activity in the "Evening," or during lights-off, than they did in the night. This is in line with previous research that has shown that male flies tend to have a steady level of activity during the night with low levels during the day, while female flies tend to have more locomotor activity during the day than the night (Tartaroglu & Emery, 2014). Interestingly, the amplitude of the transition peaks appeared to slightly increase over the first two cycles, and then slightly decrease for the last four cycles.

Similarly, flies exposed to 100 μg/mL lead displayed similar activity patterns to the control groups with rhythmic activity in the "Morning" and "Evening." They also displayed more activity during lights-off compared to lights-on (Fig. 2, 3). The lead flies also showed a slight increase in activity in the transition periods for the first two cycles and then a decrease in activity for the rest of the experiment. In contrast to the control group, however, the lead flies revealed a statistically non-significant but positive correlation between lead concentration and change in locomotor activity at masking transition periods (Fig. 4). Although this finding is not statistically significant, there still appears to be a noticeable difference between the rate of change of the two datasets at the masking transition periods.

Based on the results of this experiment, future directions could potentially offer further insight into the effects of lead on circadian rhythms in *drosophila*. It is necessary that future studies attempt to replicate the data from this experiment on a larger scale with more flies in the control and lead group. One of the limitations of the present study was that only 20 flies were used during each control and lead run, and it is therefore difficult to determine if this effect is present in a broader population. In addition, future research could explore the effects of chronic exposure to a wider range of lead concentrations. Preliminary data of the flies exposed to 75μg/mL appears to reveal a more drastic trend when compared to the control flies(Fig. 5). It appears that the activity at the transition masking periods decreases at a faster rate than that of the control flies. It is highly recommended that further testing determine if this effect is significant.

Another avenue for future testing would be to test both lead and control flies in constant darkness. This would test the strength of the circadian rhythm in drosophila flies without light stimuli, to see if lead has a noticeable effect. Circadian rhythms are endogenously generated and

normally would persist even in the absence of light, so a gradual breakdown of the circadian rhythm patterns of lead flies in constant darkness would be significant.

Due to the prevalence of lead globally, it is incredibly important for the impacts of lead on biological systems to be understood. Although certain regulations are in place, many communities are still at risk of exposure to toxic levels of lead. Knowing the effects of lead exposure could help inform policy and provide treatments for areas most affected. This study was a useful glimpse into the impact of chronic exposure to lead on the circadian rhythm patterns of *drosophila*. Accordingly, it is necessary for future research to replicate this study's findings and perhaps explore more about the toxicological impacts of lead poisoning.

5. Acknowledgements

We sincerely thank Professor Rhea Datta for giving us the opportunity to work on this project and her guidance throughout the research. We also thank Professor Herman Lehman for providing us with the activity monitor used for preliminary data collection for this experiment and for his helpful advice. Lastly, we would also like to thank the Levitt Center for their generous funding for this summer research project.

Works Cited

Aykin-Burns N., Laegeler A., Kellogg G., Ercal N. (2002). Oxidative effects of lead in young and adult Fisher 344 rats. *Archives of Environmental Contamination and Toxicology*, *44*(3), 417–420. https://doi.org/10.1007/s00244-002-2023-4.

Frank, J. J., Poulakos, A. G., Tornero-Velez, R., & Xue, J. (2019). Systematic review and meta-analyses of lead concentrations in environmental media reported in the United States from 1996 to 2016. *The Science of the Total Environment*, *694*, 133489. https://doi.org/10.1016/j.scitotenv.2019.07.295.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary toxicology*, *7*(2), 60–72. https://doi.org/10.2478/intox-2014-0009

Mirzoyan, Z., Sollazzo, M., Allocca, M., Valenza, A. M., Grifoni, D., & Bellosta, P. (2019). Drosophila melanogaster: A model organism to study cancer. *Frontiers.* https://doi.org/10.3389/fgene.2019.00051

Tataroglu, O., & Emery, P. (2014). Studying circadian rhythms in Drosophila melanogaster. *Methods*, *68*(1), 140–150. https://doi.org/10.1016/j.ymeth.2014.01.001

Vitaterna, M. H., Takahashi, J. S., & Turek, F. W. (2001). Overview of circadian rhythms. *Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism*, *25*(2), 85–93.

Wani, A. L., Ara, A., & Usmani, J. A. (2015). Lead toxicity: a review. *Interdisciplinary toxicology*, *8*(2), 55–64. https://doi.org/10.1515/intox-2015-0009.