

## Assessment of Lead Toxicity Using *Drosophila melanogaster* as a Model

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### Abstract

**Objectives:** Lead is recognized as a serious pollutant on the basis of its persistence, toxicity, bioaccumulation and extensive use in industry. Exposure to lead results in a number of cytogenetical effects on freshwater biota which adversely affects the population, including reducing the rate of cell division and inducing mutations. In order to assess the toxicity of lead, a reliable model of *Drosophila melanogaster* was taken and experiments were carried out by maintaining a control set and experimental sets with varied concentrations of lead.

**Methods:** The toxicity of lead was studied by varying the lead concentrations from 20-60 ppm in the food media that was given to the fruit flies. Studies on the larval, pupal and adult stages of the flies were done, including the reproductive and locomotion efficiency of the flies.

**Results:** The results of our study shows that as the concentration of lead in the media increased from 20 to 60 ppm, there was a visible change in the number of offsprings and the locomotive behavior of flies. Significantly there was a stretch in the time duration in the conversion of larva to pupa and then pupa to adult. The length and width of the pupa was also found to be affected.

**Conclusion:** Long term exposure to lead can have significant effect on the survival of population even if its concentration is low and it may also reflect population adaptive capacity.

**Keywords:** *Drosophila melanogaster*; Lead toxicity; Model organism; Carcinogen

### Introduction

Human activities like manufacturing, mining and fossil fuel burning has resulted in accumulation of lead in living organisms which causes various diseases like loss of appetite, headache, hypertension, abdominal pain, renal dysfunction, fatigue, sleeplessness, arthritis, hallucinations and neuro degenerative disorders. According to the Environmental Protection Agency (EPA), lead is reported as a carcinogen [1-4]. The term "heavy metals" is used for both metals and metalloids, which have high density (greater density than 4g/cm<sup>3</sup>) [5,6] and even in its low concentration it is poisonous to the organism [7]. Its chemical structure and properties are of more concern than its density. Heavy metals affecting the organism are considered along with the environmental factors such as the availability of nutrition, temperature, pH, moisture etc. [8]. Exposure to metals can occur through a variety of routes. Metals may be inhaled as dust or fume (tiny particulate matter, such as the lead oxide particles produced by the combustion of leaded gasoline). Some metals can be vaporized (e.g., mercury vapor in the manufacture of fluorescent lamps) and inhaled. Metals may also be ingested involuntarily through food items. The amount that is absorbed from the digestive tract can vary widely, depending on the chemical form of the metal and the age and nutritional status of the individual. Once a metal is absorbed, it distributes in the tissues and organs. Excretion typically occurs primarily through the kidneys and digestive tract, but metals tend to persist in some storage sites like liver, bones and kidneys for years.

These heavy metals include lead, cadmium, zinc, mercury, arsenic, silver, chromium, copper, iron and the platinum group elements. In this study, the toxic effect of lead on *Drosophila melanogaster* was evaluated. Lead is the fifth most widely used metal after iron, aluminum, copper and zinc [9]. Lead is generally introduced through the respiratory and gastrointestinal (GI) tract [10]. Once lead is absorbed 99% of it binds to the erythrocytes (RBCs) and later it is stored in the bones [10,11]. Later it can be released into the bloodstream when the exposure of lead is not there [12]. Inorganic lead is not metabolized by the hepatic metabolism and is excreted unchanged, mostly in the urine [13]. Organic lead is metabolized in the liver by cytochrome p450 dependent monooxygenase system [14] and is excreted in the form of nails and sweat [15,16]. Depending on the particle size, lead can also enter through lungs. While organic lead is well absorbed through the skin, inorganic lead is not. Since lead is chemically similar to calcium, body handles it like calcium and in the body, lead could be distributed throughout bones, teeth, liver, lungs, brain and spleen; bone being the major accumulator. Lead can cross blood brain barrier as well as placental barrier. Excretion occurs through urine and faeces. Dose and duration dependent genotoxic effects have been observed [17]. While a blood lead concentration of 10µg/dL is the current benchmark used to diagnose lead poisoning, there is ongoing research and some evidence that intellectual deficits may occur with lead concentrations as low as 7.5 µg/dL [18]. The diverse clinical manifestations can be explained by the cellular mechanisms of lead toxicity that interfere with a variety of functions, including cell membrane integrity, neurotransmitter function, heme synthesis and mitochondrial oxidative phosphorylation. Lead also causes irreversible neurobehavioral damage in many developing

mammals that increases oxidative stress which in turn leads to genetic manipulation, damage in neuronal DNA inducing apoptosis and Alzheimer's disease. Over the past few years, whole-animal approaches to study human diseases have gained more impact [19].

In the present work, the after effects of lead exposure have been studied on *Drosophila melanogaster*. *Drosophila* is a small fly, typically pale yellow to reddish brown to black, with red eyes. *Drosophila melanogaster*, the common fruit fly, has been used for genetic experiments since T.H. Morgan started his experiments in 1907. Many species including the noted Hawaiian picture wings have distinct black patterns on the wings. The plumose (feathery) arista, bristling of the head and thorax, and wing venation are characters used to diagnose the family. Most are small, about 2-4 mm long, but some, especially many of the Hawaiian species, are larger than a house fly. Here fruit fly is considered as a model organism since it is cost efficient, breeds quickly, growth acceleration is easy, undergoes genetic manipulation rapidly and has identical match with human diseases [20]. *D. melanogaster* is a complex multi-cellular organism in which many aspects of development and behavior parallel those in human beings (Figure 1). They are small have a life cycle of less than two weeks (short generation time) and grown on simple media (oatmeal/yeast/molasses/banana), single male and female can produce more than 100 progeny (high reproductive rate) [2], and ability to perform large-scale genetic and/or compound screens, which cannot be developed in other higher animal models due to cost, time and legal or ethical boundaries and issues.

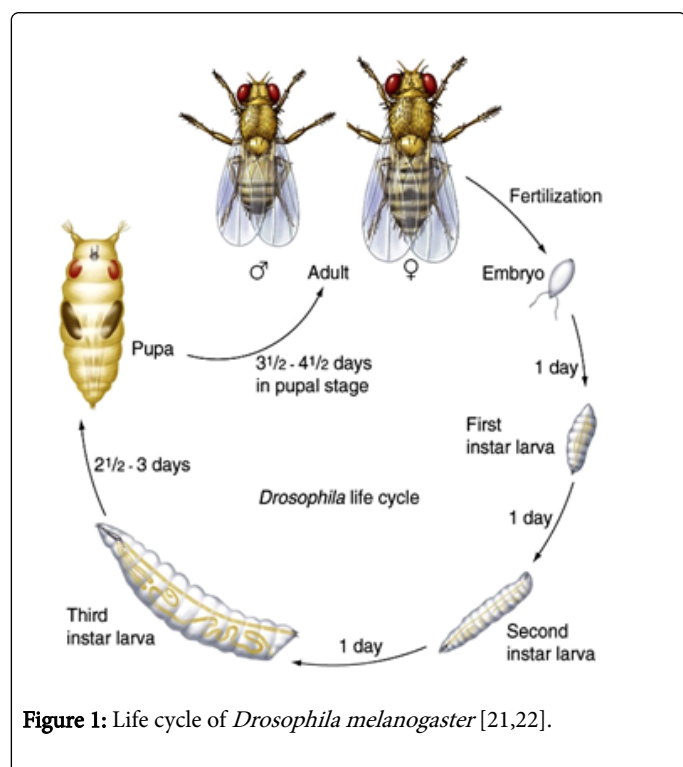


Figure 1: Life cycle of *Drosophila melanogaster* [21,22].

In a recent compilation, 61% of human disease genes were found to have apparent homologs in *Drosophila melanogaster*. This conservation suggests that flies have a bright future as a model for understanding many human genetic diseases [23,24]. Not only as a disease model but also novel events at molecular level and key contrivances of distinctive glial insusceptible responses were also studied using fruit flies [25]. In the recent times, researchers have

studied the after effects of cigarrate smoke on the epithelium of fruit flies [26], whereas in another study the effect of volatile organic compounds given out by growing fungus molds on flies was done [27].

## Materials and Methods

### Collection of *Drosophila melanogaster*

The wild strain of *Drosophila melanogaster* was obtained from the Department of Zoology, University of Mysore and the flies were bred and allowed to multiply in a clean container that was regularly monitored every 6 h. Later flies were collected, identified and separated as male and female flies. This was done on the basis of size, color of abdomen and pattern of hair growth on the body. The females were about 25% larger than the males. On a male fly, the last two segments of the abdomen were much darker than the female. The males have thick black bands with a rounded abdomen, whereas the females tend to have one dark band with a pointed abdomen.

### Media preparation

The first step in rearing the flies is the preparation of media. Wild-type flies were used in this study. They were kept in flask with commonly used artificial medium that was made by using 25 g of wheat cream and jaggery, 2.5 g of agar and 1.875 ml of propionic acid. These constituents were boiled in one liter of distilled water till a creamy consistency was reached. Here propionic acid acts as anti-fungal/bacterial agent. Once the media cools down a little, it is poured into sterilized bottles and plugged with sterile cotton. Few granules of baker's yeast is added to the medium and then the preparation was transferred to four separate sterilized bottles out of which one was control set and the other three experimental sets (Figure 2).



Figure 2: Control and Experimental sets.

### Preparation of control and experimental sets

In order to transfer the collected flies it has to be anesthetized first. The least harmful method for anesthetize is either carbon dioxide or cooling (freezing) anesthetizing. Out of these choices cooling is simplest requiring freezer and ice cubes. Lead nitrate was used as a source of lead in the media. During the cooling method to immobilize the flies, culture bottles were kept in freezer for 20 min, and when the flies were anesthetized they were transferred to bottles containing media (control set) and media induced with lead (experimental set up). One pair of each male and female fruit fly was transferred into the four bottles for the purpose of breeding. Lead in the range of 20, 40 and 60 ppm was introduced in the media that was poured into the experimental set of bottles. After sometime the flies were out of anesthetize effect and woke up. In addition it is the only method which

will not affect the structure and neurology of the flies. The behavior of flies can be studied after they have warmed up. The transferred flies were allowed to mate in the bottles. Generally male flies will mate if they are 3 to 5 days mature. Mating occurs quickly and females lay eggs soon after mating. In each group set of experiments, 10 flies were used.

### Assessment of morphological changes

In the control set of flies, where there was no lead introduced into the food dosage the normal larval period was around  $105 \pm 0.5$  h and the pupal period was approximately  $82.4 \pm 0.3$  h. The rate of conversion of larva to pupa and later pupa to adult was 99%, whereas the egg hatching percentage was above 78%. The larval and pupal period was studied in terms of the duration of time and the conversion rate of larva to pupa and pupa to adult was also observed. The percentage of egg hatching was also a factor in assessing the toxicity of lead.

### Assessment of pupae size

Size of pupae of *Drosophila melanogaster* was studied using Vernier calipers and this was performed for the control set as well as for all the varying concentrations of lead in the experimental sets (Figure 3).



Figure 3: Pupa on the surface of the bottle.

### Assessment of reproduction rate

The rate of reproduction of the flies was also studied for the control set and for all the varying concentrations of lead in the experimental sets.

### Assessment of locomotive activity

This was done in two ways. Firstly, by placing around 10 flies at the bottom of 25 mm diameter tube (Figure 4) and then recording the climbing behavior of the flies up to a distance of 10 cm and more. Secondly, by keeping one fly inside each under control and experimental set of petriplate to assess the moving behavior [28]. The locomotion was studied for flies under each set of lead concentrations as well as for the control set. These studies were carried out in triplicates.



Figure 4: Locomotive activity of flies.

## Results and Discussion

### Assessment of morphological changes

The result shows the presence of lead in the medium increased the larval and pupal period, but decreased the rate of larvae transforming into pupa, pupa to adult, and the percentage of eggs hatching at a specific time period. Lead after being swallowed induced its toxic effects on the body of larva and decreased its growth, delaying the development of the fly. The authenticity of this claim is the significant difference of the average length of larvae between the control group and groups exposed to different concentrations of lead (Table 1).

Lead nitrate concentration (ppm)	Larval Period (hour)	Pupal Period (hour)	Conversion rate of larva to pupa %	Conversion rate of pupa to adult %	Egg hatching %
20	$155.9 \pm 1.8$	$97.4 \pm 1.6$	89.5	83.6	48.4
40	$174.8 \pm 1.3$	$99.5 \pm 1.8$	80.8	64.4	37
60	$252.3 \pm 2$	$101.3 \pm 1.4$	51	36.4	24.8

Table 1: Quantitative Morphological Changes.

### Assessment of pupae size

Lead can also affect the fetus inside the egg and may cause delay in larva emergence from the eggs. To illustrate that, a significant lead concentration dependent delay, was occurred in the onset of larval sampling, the control larvae had reached the stage of pupation. In this

study we used the fruit fly as a model for developmental studies. Figure 5 represents the morphological changes taking place in the fruit fly during its development.

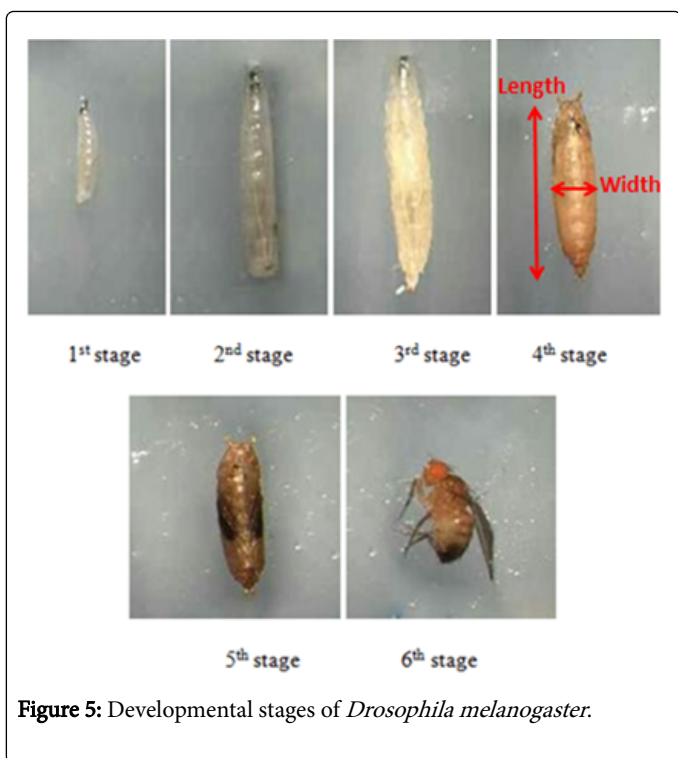


Figure 5: Developmental stages of *Drosophila melanogaster*.

In the control set of flies, where there was no lead introduced into the food dosage the average length of pupa was found to be 3.5 mm and the average width was 1.1 mm.

After reviewing the results, it can be seen that with increased concentration of lead, the rate of larvae transforming into pupae and pupa to adult reduced in a nearly direct correlation to the concentration of lead in the medium. However, probably due to the insect's ability to detoxification of the metal at lower concentrations, there is no discernable negative effect on the pupa, but in higher concentration, probably the Metallothionein (MT) protein is incapable of detoxification. Also, high concentrations of this metal can lead to impaired expression of MTs, resulting in the accumulation of large metal deposition in the body, inducing deleterious effects on the larva. As a result, there is a significant decrease in the survival potential of larvae and the rate of larva transforming to pupae decreases (Table 2).

Lead nitrate concentration (ppm)	Average length of pupa(mm)	Average width of pupa(mm)
20	3.1	0.9
40	2.9	0.85
60	2.5	0.79

Table 2: Effect of lead concentrations on the length and width of pupa.

### Assessment of reproduction rate

The reproduction rate had increased with the introduction of minute lead concentration and decreased proportionally as the concentration of lead increased in the media (Figure 6). In general, results show that the increased concentrations of lead critically affect the various stages of development in the fruit fly. The toxicity of lead reduced the fertility and egg hatching. So it can be claimed that the toxicity of lead would result in a negative effect on the viability and developmental stages in the organism. It also can be said that the presence of 20 ppm of lead nitrate in the media had the least effect on the fly. The count of the total off springs was taken only for the fully formed adult flies. The dead pupa or larva was not counted.

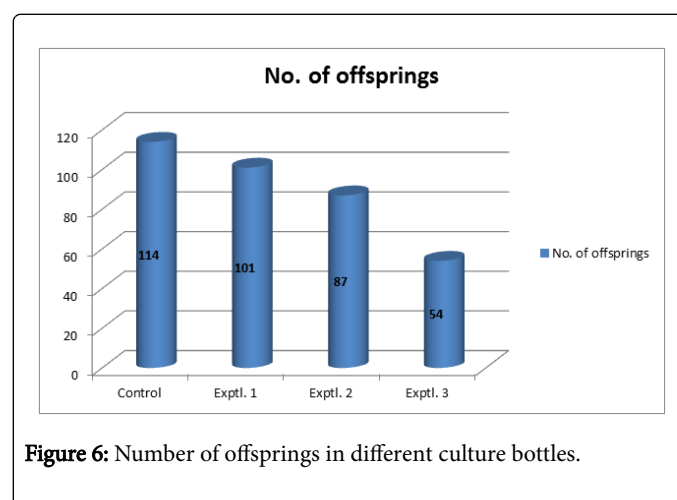


Figure 6: Number of offsprings in different culture bottles.

Given that fruit fly has a relatively high resistance to the lead; we cannot assume this insect as an indicator to assess the lead presence in the environment. However, this insect can be as an appropriate model to examine the effect of lead on the developmental stages of an organism.

### Assessment of locomotive activity

Active locomotion of *Drosophila melanogaster* was seen more in the minor concentrations of lead, whereas the activity was found to reduce and the locomotion of flies was observed to decrease significantly when the concentration of lead was increased in the media. The first set of studies revealed that in the control set, the flies were able to

climb the walls of the tube easily, whereas this was not the case with the experimental tubes that contained lead in the food dosage. To know if there is any statistically significant difference between the groups, t-test was done using SPSS 15.0 descriptive statistical tool [29] and observations were made as shown in Table 3.

Lead nitrate concentration (ppm)	Mean	N	Standard deviation	Standard Error Mean
20 ppm	9.5000	10	1.35401	0.42817
40 ppm	5.6000	10	0.69921	0.22111
60 ppm	2.3000	10	0.48305	0.15275

**Table 3:** Statistical significance among the groups.

In the second set of studies, the movement of the flies has been recorded as shown in (Figure 7).



**Figure 7:** Distance covered by *Drosophila melanogaster* (from L to R- Control set, Expt. Set 1, 2 and 3).

The green dot represents the starting point whereas the red dot represents the end point of the movement of flies (Table 4). The flies which were untreated with lead covered a minimum of 28 cm and a maximum of 123 cm in less than 15 seconds.

Sets of bottle	Distance covered in 15 sec (cm)
Experimental set 1	12.5
Experimental set 2	6.2
Experimental set 3	3.7

**Table 4:** Studies on locomotion.

### Conclusion

In our study we have demonstrated an easy method to study the after effects of lead treatment in *Drosophila melanogaster*. The methods can be applied to any detailed investigation in this organism where metabolite observations are required to underpin genetic and other pathophysiological manipulations. The results indicate that an increased concentration of heavy metal (lead) is effective in the developmental stages of the fruit fly. In insects that spent their embryonic and maturation period in a medium containing lead and fed there, their hatching percentage decreased significantly. In relatively high concentrations of lead ions, developmental processes face severe imbalance in the process of conversion from larval to adult

stages. Thus, *Drosophila sp.* continues to provide an intriguing system for metabolomics studies and should be more widely exploited.

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