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Bisphenol A (BPA): An Endocrine Disruptor Affecting Behavior and Metamorphosis

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Abstract

Contamination of environment by toxic pollutants has become commonly recognized as an environmental concern. These compounds are often present at low concentrations making them difficult to detect, while they cause ecological issues according to their nature (heavy metals, pesticides, endocrine disruptors, etc.). Therefore, ecotoxicity risks due to these chemicals can be evaluated using various protocols i.e. from cell-lines and microorganisms to plants, invertebrates and vertebrates. Most of them allow the detection of a phenotype, thus indicating a toxic effect and some of them can also highlight the modification of molecular actors involved in the toxic response. However, few animal models offer the opportunity to perform integrated study with multiple approaches, from molecular variations to physiological consequences. *Drosophila melanogaster* is a proven model organism in genetic and biology research in the past few years and can be considered as an emerging and suitable model to study toxicology and ecotoxicology. Drosophila has a short and well described development; reproduction and its maintenance does not require sophisticated equipment and are rather economical. Moreover, the fruit fly's development is under hormonal control making endocrine disruptors studies suitable in this model. Hence, to develop this “tool box” as an alternative model, this study was aimed to analyze the development and behavioural changes in *Drosophila melanogaster* to the exposure of bisphenol A (BPA).

Keywords: Bisphenol-A, *Drosophila melanogaster*, behaviour, metamorphosis, endocrine disrupters.

1. Introduction

Humans and all animals on this globe are exposed to a great and diverse number of external substances in their respective environments. The exposure to these natural or synthetic compounds could alter many physiologic processes during development and/or adult life. Specifically, exposure to such compounds may impair the physiology, reproductive and immune system. A particular group of substances are called endocrine-disrupting chemicals (EDCs). The term "endocrine disruption" describes how outside variables, such as chemicals

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or other environmental agents, interfere with or modify the endocrine system's regular functioning. According to Kraak et al (2001), these disturbances may result in several detrimental consequences for the organism, such as imbalances in hormones, developmental disorders, and sexual abnormalities. EDCs are defined as chemicals that may interfere with the body's endocrine system inducing adverse developmental, reproductive, neurologic, and immune effects in both humans and wildlife (del-Mazo et al 2013). To date, hundreds of compounds, and thousands of others suspected to having similar properties have been identified as EDCs. EDCs include a very broad repertoire of natural and man-made substances found in daily products, such as plastics, food bottles, food cans, detergents, flame retardants, toys, cosmetics, and pesticides. Studies in human populations are limited, but there has been a plethora of studies performed in cells and animal models that suggest the potential adverse effects of EDCs in human health (Sweeney et al 2015; Wilhelm et al 2015; Latini et al 2010)

Drosophila melanogaster, the fruit fly, has emerged as a significant model organism contributing to various scientific disciplines. Due to its conserved biological pathways, well-characterized genome, and flexible genetic tools, *Drosophila melanogaster* continues to be a widely used model organism to understand intricate genetic phenomena such as gene interactions, evolutionary genetics, and the molecular mechanisms underlying development. It is an invaluable tool for analysing gene functions and comprehending the genetic underpinnings of human diseases due to its extensive mutant collections and genetic manipulation capabilities. Furthermore, the development of vital resources and databases that support genetic studies has been made easier by the highly collaborative nature of the Drosophila research community (Li et al., 2022). The use of *Drosophila melanogaster* as a research model to study human neurodegenerative diseases is one of its many noteworthy contributions. The insights gained into the molecular basis of neurodegenerative diseases using *Drosophila melanogaster* can guide therapeutic research and the development of potential treatments as illustrated in Figure 1.

Entomologists have been increasingly concerned in recent years about the effects of pesticides on insects that manage to survive exposure to harmful chemicals (Knutson, 1959). The development of pesticide resistance in specific insects has been the subject of numerous studies; however, the effects of newly developed insecticides and their potential to disrupt insect endocrine systems have received relatively little attention (Pittendrigh et al., 2014). However, over the past 15 years, several studies have been published that provide insight into the sublethal effects of pesticides on beneficial arthropods, such as *Drosophila melanogaster* and honey bees. According to these studies, among other sublethal effects; pesticides can affect these insects' behavior, neurophysiology, and ability to learn (França et al., 2017). Specifically, some of these studies have investigated the possibility of endocrine disruption in the model organism *Drosophila melanogaster.* Yılmaz et al. (2020) stated that changes in brain morphology and neurodevelopmental impairments have been linked to EDCs. Given that humans and *Drosophila melanogaster* share many basic biological processes, it is a valuable model for studying how EDCs affect brain morphology and neurodevelopment.

Research has developed comprehensive *in vivo* procedures to examine how EDCs affect Drosophila fecundity/fertility, developmental timing, and lifespan (Brander et al 2026). The effects of exposure to different EDCs, like bisphenol A (BPA) and 17-α-ethinylestradiol (EE2), have been evaluated using these characteristics. The regulation of growth and maturation during juvenile larval life as well as adult behavioural and metabolic adaptation have been studied with the endocrine mechanisms of environmental adaptation in *Drosophila melanogaster* (Kilbourn et al 2026). The present work is an extension to the notion that BPA is a potent endocrine disruptor that not only impact fertility and reproductive processes but also disrupts normal physiological functioning in *D. melanogaster*.

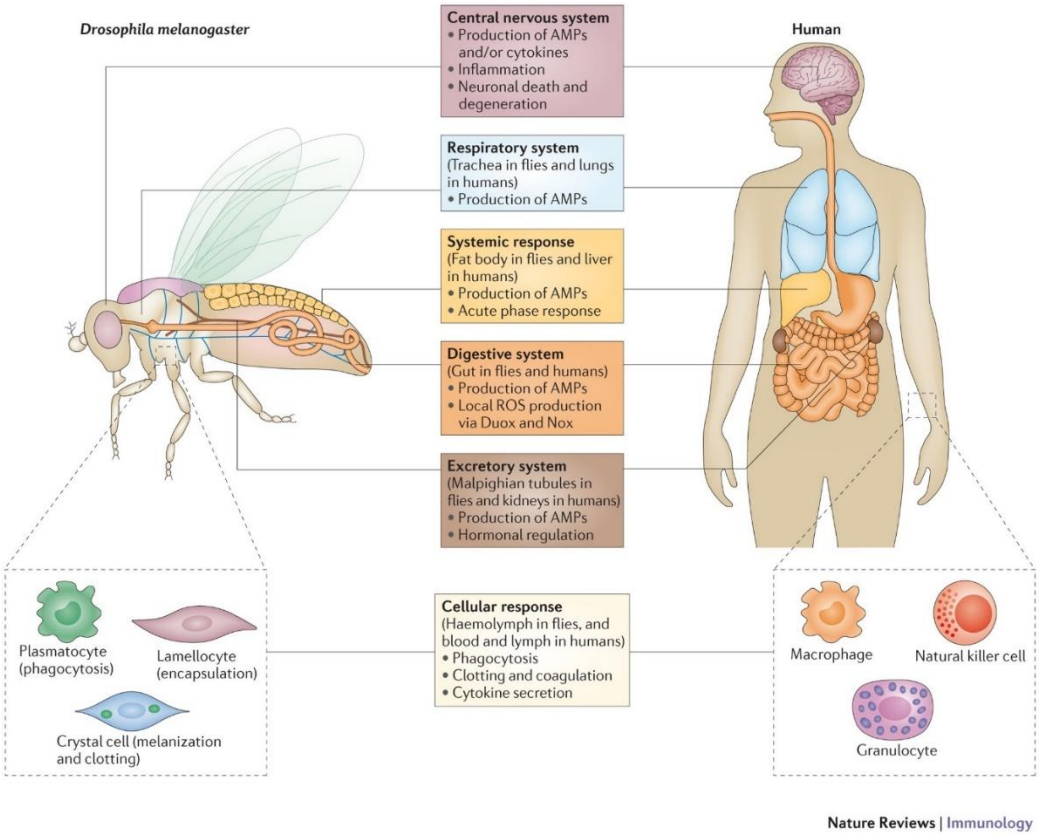

Figure 1: Drosophila and its inter-relationship with humans (Source: Buchon et al. 2014).

2. Methodology

2.1 Preparation of fly food

Standard fly food was prepared using ingredients like agar, sugar, yeast, and cornmeal, to provide the flies with essential nutrient requirements (**Table 1**). To facilitate homogeneous dispersion, agar was first dissolved in distilled water and heated. Once completely dissolved, the mixture was allowed to cool to around 50°C. Following that, yeast, corn starch, and sugar were sequentially incorporated and carefully mixed to ensure uniform distribution and prevent lump formation. The entire fly food mixture was then transferred into sterile glass bottles and allowed to solidify for at least 48 hours at room temperature. The goal of this standardized food preparation protocol was to provide a uniform and uncontaminated food source for the *Drosophila melanogaster* cultures throughout the experiment.

Table 1: Standard composition of Cornmeal food for *Drosophila melanogaster*

SUGAR	88g
CORN	80g
YEAST	21g
AGAR	6g
SODIUM BENZOATE	0.8g
PROPIONIC ACID	10ml
WATER	700ml

2.2 Maintenance of drosophila cultures

Bottles containing Drosophila are carefully maintained within a controlled Biological Oxygen Demand (BOD) chamber at a constant temperature of 25°C. Recognizing the importance of the circadian rhythm in regulating various physiological and behavioral processes in *Drosophila melanogaster*, a strictly controlled 14-hour light/10-hour dark cycle was implemented within the BOD chamber. This cycle mimicked natural environmental conditions, allowing flies to synchronize their internal biological clocks with the external light-dark cycle. Other environmental parameters, such as humidity and ventilation, were also monitored and kept within optimal ranges in addition to temperature and light regimes. The BOD chamber was cleaned and disinfected regularly to reduce the risk of microbial contamination, which could harm fly health and experiment outcomes. Consistent synchronization reduced variability in gene expression, hormone levels, and behavioral patterns, improving the reproducibility and reliability of experimental data. The flies might need to be anesthetized before certain procedures, like transferring flies or gathering virgin females, can be completed. Diethyl ether is usually used for this, and tiny painting brushes were used to handle the flies.

2.3 Bisphenol A (BPA) exposure and dose preparation

To investigate the effects of BPA on *Drosophila melanogaster*, four distinct concentrations of BPA were prepared: 0.1 mM, 0.25 mM, 0.5 mM, and 1 mM, as well as a control group. For precise dosage control, high-purity (>99%) Bisphenol A (LOBA Chemie, product number 0218300500) was used. BPA was crushed to make powder and dissolved in water to make a 1mM stock solution due to its limited solubility in water. To guarantee a consistent dispersion of BPA throughout the substrate, dilutions of this stock solution were added to the standard Drosophila food mixture. Following thorough mixing, the substrate with the corresponding BPA concentrations was dispensed into equal-volume sterile glass vials. Each vial was labelled with its concentration and allowed to solidify at room temperature for at least 48 hours. Drosophila cultures were established within the vials following food preparation. To achieve the desired 3:1 female-to-male ratio, virgin female flies (n = 6) were

carefully selected and paired with two male (n=2) flies in each vial. This process of selection and transfer ensured controlled mating and lower undesirable variability in offspring production. For each BPA concentration and the control group, triplicate sets of vials were established, resulting in a total of (3 exposures × 4 concentrations + 1 control) × 3 replicates = 39 experimental units. The vials were then placed in a controlled environment chamber with a constant temperature of 25°C and a 14:10 light-dark cycle. This cycle replicated natural environmental conditions and synchronized the flies' internal circadian rhythms, increasing reproducibility while minimizing stress-related challenges.

Throughout the experiment, observations and data collection was carried out; the larvae and flies were manually counted and transferred. To reduce the impact on the circadian rhythm, the counting was done between 12:00 pm and 4:00 p.m. The entire experiment lasted for four months.

2.4 Assays for Investigating the Effects of Endocrine Disruptors on Drosophila melanogaster

2.4.1 Eclosion assay- *Drosophila melanogaster* was maintained in standard conditions. Four different concentrations of BPA (0.5mM, 0.25mM, 0.1mM, and 1mM) were given to the Drosophila in a 3:1 (female-to-male) ratio. The number of pupae was manually counted and recorded. The development from pupa to adult was monitored and recorded. The eclosion rate was calculated by dividing the number of adults by the total number of pupae for each BPA concentration.

2.4.2. Larval Locomotory Assay- The first behavioral assay conducted was the Larval Locomotory Assay. A fine layer of 10% yeast suspension was placed over a 2% agarose gel layer that had been prepared in a petri dish with a diameter of 15 centimetres. Larvae in their third instar were obtained for each concentration and control was placed in the middle of the agar plate. To avoid the attachment of food particles to the larva, which may reduce its movements, the larva was first placed into 2 or 3 drops of water in a separate petri dish before being transferred to the agar-coated plate. After a 1-minute acclimatization period, the path of the larva was traced and observed for every minute on a transparent paper manually using a marker pen. The path of the larva was then measured in centimetres using a thread.

2.4.3. Drosophila Climbing Assay- The second behavioral assay conducted was the Drosophila Climbing Assay. Newly emerged sexually naive flies (3 males and 3 females) from each concentration and control were taken. The flies were placed in two test tubes, which were joined with the help of tape and parafilm to form a long tube of 15 centimetres. A 5 cm threshold was marked for each fly. With a concerted effort to push the flies to the inner bottom, the test tubes were repeatedly tapped against a closed cell foam. The total number of flies that crossed the threshold line of 5 cm in 10 seconds was counted manually. To count the flies that had climbed, video recordings were also made, and any leftover pheromones were removed from the test tubes by rinsing them with 95% ethanol.

3. Results

3.1. Eclosion assay: This study was done to see the effects of different concentrations of the

endocrine disruptor (BPA) on the life cycle of *Drosophila melanogaster*. The life cycle stages were observed at regular intervals and the results are presented in **Figure 2**. The life cycle of the control group followed the expected pattern, with adult *Drosophila* emerging on the 12th day. At 0.1 mM BPA **e**xposure, delayed the development of the third instar and prepupa stages by 24h was noted as compared to the control group. However, the emergence of the adult was not delayed**.** At 0.25 mM BPA and 0.5 mM BPA exposure, the development of the larvae did not delay significantly but delay in the development of the prepupa and pupa stages occurred by one day (24h) as compared to the control grou

p. However, at 1 mM BPA exposure prepupa and pupal stages got delayed by 2 days compared to the control group and the emergence of the adult happened on day 14th. Though it is clearly noticed that most of the pupae emerged into adulthood, exposure to BPA resulted in delayed metamorphosis (**Table 2**). These results suggest that exposure to BPA can delay the development of *Drosophila melanogaster*, particularly the transition from larvae to prepupa and from prepupa to pupa. The delay in development became more pronounced at higher concentrations of BPA, with the emergence of the adult also being delayed at the highest concentration tested (1 mM). These findings highlight the potential impact of endocrine disruptors on the development and life cycle of fruit flies.

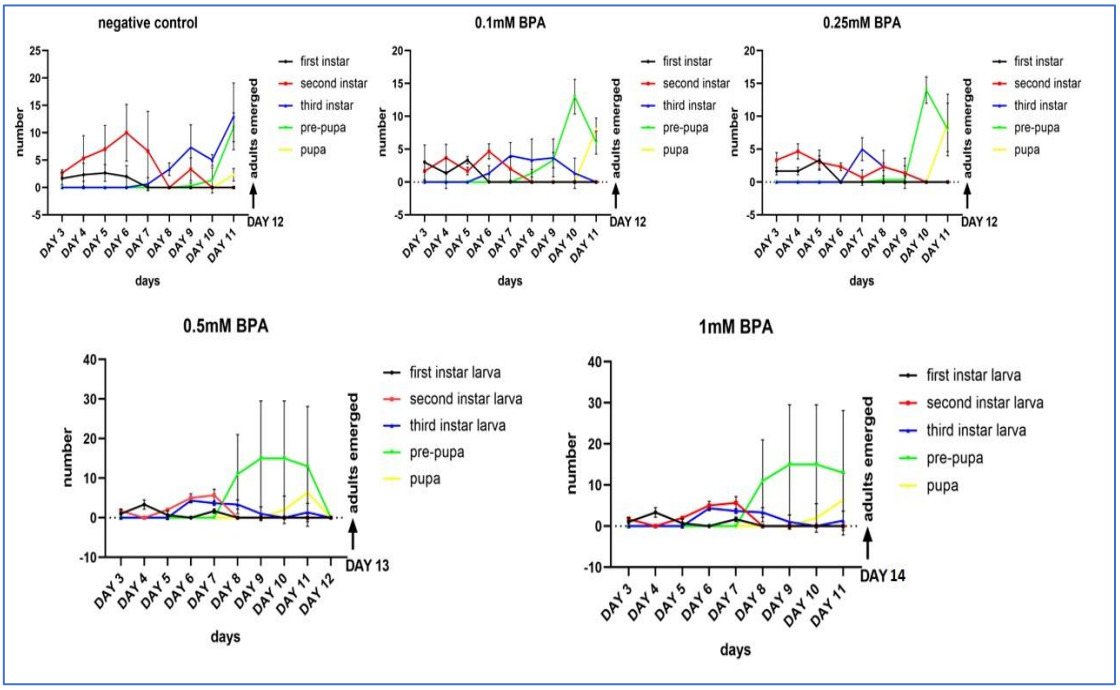

Figure 2: Eclosion assay at different treatment with Bisphenol A (BPA) showing the life stages and emergence to adults of *Drosophila melanogaster* at different days.

Table 2: Shows the average pupae and the emergence to the adults on exposure to different concentration of BPA

BPA Concentration	Average Pupa Count (triplicate)	Conversion to Adults
1mM	7	90%

0.5mM	6	~100%
0.25mM	9	~100%
0.10mM	8	~100%

3.2. Larval Locomotory Assay: The results of the Larval Locomotory Assay revealed a significant impact of BPA on the locomotion of the larvae, particularly in females. While 1 mM concentration of BPA affected both males and females, all concentrations of BPA had a more pronounced effect on females. This is likely due to BPA's properties as a xenoestrogen, which primarily affects female endocrine and reproductive systems as shown in **Figure 3.**

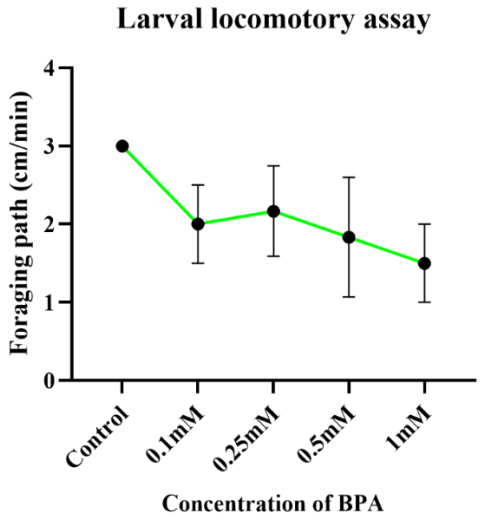

Figure 3: Graph showing Locomotor Performance of *Drosophila melanogaster* at different BPA cconcentrations.

3.3. Drosophila Climbing Assay: The *Drosophila* Climbing assay, also known as the Negative Geotaxis Assay, showed that at 1 mM concentration, BPA significantly affected larval locomotion, particularly in females as compared to other concentrations (**Figure 4**).

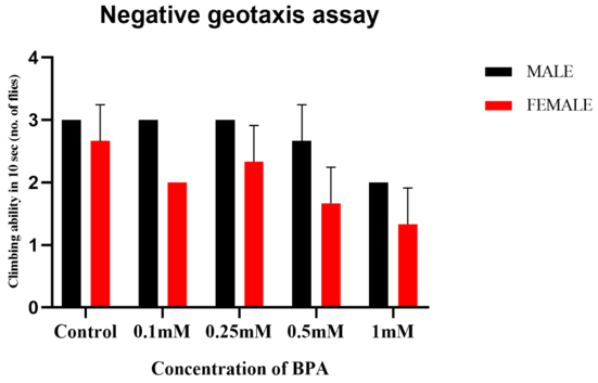

Figure 4: Graph Showing the Climbing ability of *Drosophila melanogaster* at different concentration of BPA treatment.

Discussion

This study provides a comprehensive examination on the effects of Bisphenol A (BPA), a widespread endocrine disruptor, on the developmental and behavioral aspects of *Drosophila melanogaster*. Our findings align with and extend the existing body of literature in several key ways; as these findings are consistent with the observations made by Begum et al. (2021), further validating the impact of BPA on the behavior of *Drosophila melanogaster*.

Firstly, our observation that BPA exposure significantly delays the larval-pupal development of *D. melanogaster* corroborates the findings of Janner et al. (2021) who reported a decrease in the rate of pupal eclosion and lifespan of hatched flies. This is further supported by Wang et al. (2025) who found that BPA exposure delayed larval development and affected axonal growth in *D. melanogaster*. However, our study advances this understanding by demonstrating that the prepupa-pupa transition is particularly sensitive to BPA exposure.

Secondly, we found a sex-specific decrease in female locomotor speed upon BPA exposure. This aligns with the findings of Begum et al. (2020) who reported a significant reduction in climbing ability in female *Drosophila* exposed to a higher concentration of BPA. Interestingly, Izumi et al. (2008) also found that BPA exposure resulted in a lower number of females in the housefly *Musca domestica*, suggesting that BPA may have broader impacts on female insects.

Contrary to Weiner et al. (2014), who found that BPA exposure increased larval growth and advanced the onset of metamorphosis in flies, our study did not observe these effects. This discrepancy may be due to differences in experimental design or BPA concentrations used, underscoring the need for further research to reconcile these findings. Recent studies have shown that exposure to BPA significantly alters the behavioral characteristics of *Drosophila melanogaster*. Begum et al. (2020) examined how BPA affected various behavior in *Drosophila melanogaster* larvae, including feeding rate, foraging ability, climbing ability, and courtship display. The results showed significant decreases in the feeding rate, the length of the foraging path, and the frequency of courtship displays. Kaur et al. (2015) also reported the effects of BPA on larval locomotor parameters, including walking speed, distance travelled, mobility, turn angle, and angular velocity, as well as social behavior impairment. Researchers also examined how Bisphenol A (BPA) affected *Drosophila melanogaster's* growth and developmental trajectory in the academic study published by A.K. Weiner et al in 2014. The results of the study showed that the subject species' larval growth was increased and their metamorphic process was accelerated by BPA exposure. This emphasizes how profoundly BPA affects *Drosophila melanogaster's* physiological functions. Wang et al. (2025) investigated the effects of bisphenol A (BPA) and its derivatives on *Drosophila melanogaster* in a thorough investigation. The results showed that exposure to these substances affected the growth of axons and caused a delay in the development of larvae. In a different study, Musachio et al. (2021) examined the consequences of BPA exposure during embryonic development in *Drosophila melanogaster*. Their findings suggested that the pupal eclosion rate had decreased, which had a knock-on effect on the emerged flies' lifespan. These investigations highlighted the substantial influence of BPA on *Drosophila melanogaster* developmental processes.

Our study did not find any effects of BPA on post-embryonic development, larval weight, stage duration, or pupal length, unlike Maria et al. (2019) who studied these effects in the cotton pest *Spodoptera littoralis*. This suggests that the effects of BPA may vary across different insect species and warrants further investigation.

Our study's findings on *Drosophila melanogaster* have broader implications for understanding BPA's effects on other organisms, including humans. BPA's disruption of developmental and behavioral processes could potentially be mirrored in other species, leading to health issues such as reproductive disorders, neurodevelopmental delays, and increased risk of certain cancers in humans. Furthermore, the potential ecological impact of BPA exposure, as seen in our study, could affect ecosystem stability and biodiversity by altering population dynamics and food webs. In conclusion, our findings collectively indicate that BPA can disrupt key developmental and behavioral processes in *D. melanogaster*, potentially affecting their fitness and the resilience of their ecosystems.

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