Developmental and Reproductive Effects of Bisphenol A (Bpa) in *Drosophila Melanogaster*

Bisfenol A'nın (BPA) *Drosophila Melanogaster*'de Gelişim ve Üreme Üzerine Etkileri

Research Article

Emel Atlı [1,*], Hacer Ünlü[2]

[1] Nevşehir University, Faculty of Education, Department of Elementary Education, Nevsehir, Turkey

[2] Hacettepe University, Faculty of Science, Department of Biology, Ankara, Turkey

ABSTRACT

The aim of this study was to investigate the effects of BPA that is one of the endocrine disrupting chemicals (EDCs) on the development and reproduction of *Drosophila melanogaster*. Larvae of *D. melanogaster* were exposed to 0.1 mg/L, 1 mg/L and 10mg/L BPA. The percentages and times of transition from larvae to pupae and from pupae to adults and the mean offspring number were determined. No differences were found in the transition percentages from larvae to pupae and from pupae to adults ($p > 0.05$). However, it was found that both the mean pupation and the mean maturation times were delayed with BPA exposures ($p < 0.05$). In the 0.1 mg/L and 1 mg/L exposed groups, the mean offspring numbers were significantly less than that of the control groups ($p < 0.05$).

Keywords
Bisphenol A (BPA), *Drosophila melanogaster*, Development, Mean offspring number.

ÖZET

Bisfenol A (BPA), canlı organizlarda hem toksik hem de biyolojik etkiler gösterdiği belirlenen biridir. Bu çalışmanın amacı, endokrin bozucu kimyasallardan (EBK) biri olan BPA'nın *Drosophila melanogaster*'in gelişimi ve üremesi üzerine etkilerini araştırmaktır. *D. melanogaster* larvalarına 0.1 mg/L, 1 mg/L ve 10 mg/L BPA uygulanmıştır. Larvadan pupaya, pupadan ergine geçiş yüzdeleri ve süreleri ve ortalama yavru döl sayıları belirlenmiştir. Larvadan pupaya, pupadan ergine geçiş yüzdelerinde anlamlı bir değişim gözlenmemiştir ($p > 0.05$). Ancak BPA uygulamaları ile hem ortalama pupalaşma hem de ortalama erginleşme süreleri gecikmiştir ($p < 0.05$). 0.1 mg/L and 1 mg/L BPA uygulama gruplarında, ortalama yavru döl sayıları kontrol gruplarına göre anlamlı derecede azalmıştır ($p < 0.05$).

Anahtar Kelimeler
Bisfenol A (BPA), *Drosophila melanogaster*, Gelişim, Ortalama yavru döl sayısı.

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Correspondence to: Emel Atlı, Nevşehir University, Faculty of Education, Department of Elementary Education, Nevsehir, Turkey

Tel: +90 384 2281004 Fax: +90 384 2281015 E-Mail: eakkan@hacettepe.edu.tr

INTRODUCTION

In recent years, the potential adverse effects of endocrine disrupting chemicals (EDCs) in humans and wildlife have led to increasing public and scientific concern. Within this diverse group of EDCs, the most well known are the "xenoestrogens" which mimic the effects of the female steroid hormone estrogen via interaction with the cellular receptor [1].

Bisphenol A (BPA; 4,4'-dihydroxy-2,2-diphenylpropane; CAS 80-05-7) is one of the industrial compounds that have generated concerns due to its high production and widespread use in many consumer products [2]. BPA is used to produce epoxy polycarbonate resins, which are employed in the manufacture of a wide range of consumer products, such as food containers, and in medical applications [3]. Recent studies have shown that it can leach out of certain products, including the plastic lining of cans used for food, polycarbonate babies' bottles, white dental fillings and sealants [4-6]. It was reported that BPA is a xenoestrogen that potentially can have adverse effects on living organisms [1, 2, 7-9].

Studies on the effects of BPA have centered mainly on effects in vertebrates. From many studies reported in the literature about endocrine disruption (ED), only a minor fraction have investigated their effects in invertebrates; from these only 10% were conducted with terrestrial invertebrates [10].

Laboratory experiments detected some abnormalities in development and reproduction of various invertebrates species exposed to BPA. For example, Watts et al. [1, 9] reported that BPA exposure delayed moulting and increased mouthpart deformities in the midge *Chironomus riparius*. In another study, it was suggested that BPA delayed emergence times, suggesting some disruption of normal development in *Chironomus* [11]. Lemos et al. [3, 10, 12] found that BPA elicited developmental and reproductive toxicity in terrestrial isopod *Porcellio scaber*. The effects of BPA on the life cycle of the housefly *Musca domestica* were examined in another study done by Izumi et al. [13]. They found that both the survival ratio of eggs to the third instar larval stage and the ratio of pupae to larvae decreased and a delay in the timing of emergence typically was observed in insects exposed to > 100 μg/kg BPA.

Potential adverse developmental and reproductive effects of BPA in invertebrates needs clarification, particularly since invertebrates represent 95% of all animal species. In addition, the aquatic organisms may not be ideal reprentatives of the major invertebrate groups [1, 14]. From this point of view *Drosophila melanogaster* can be a suitable organism to determine adverse effects of these chemicals because of its short developmental cycle.

In this study, the developmental and reproductive effects of BPA were examined by taking into account the changes in developmental stages and the differences in mean offspring number in *Drosophila melanogaster*.

MATERIALS AND METHODS

The organism and environmental conditions

In this study, the wild type Oregon strain of *Drosophila melanogaster* was used. The flies were kept in a *Drosophila* culture room (Hacettepe

Table 1. The changes of the transition rate from larvae to pupae depending on BPA exposure.

Groups	Number of larvae	Number of pupae	Rate ± S.E.	S.D.	p
Control	100	96	96 ± 1.63	3.27	0.431
Acetone Control	100	96	96 ± 2.31	4.62	
0.1 mg/L (B1)	100	98	98 ± 2.00	4.00	
1 mg/L (B2)	100	95	95 ± 1.92	3.83	
10 mg/L (B3)	100	92	92 ± 2.83	5.66	

B: BPA exposure group, S.E.: Standard error, S.D.: Standard deviation, p > 0.05.

University, Ankara, Turkey) at 25±1°C and relative humidity of 50-60% and in 12 hrs light, 12 hrs dark periods on a standard cornmeal *Drosophila* medium.

Virgin Oregon females and males of the same age were crossed in culture bottles. Individuals were then removed from the culture bottles after 8 hours. 72±4 hrs later, the third instar larvae were collected.

BPA exposures

The solutions of BPA were prepared from solid compounds (Sigma-Aldrich; Steinheim, Germany). A known amount of BPA was diluted in 1 mL acetone and it was fulled to 1 liter with 5% sucrose (Merck; Durmstadt, Germany) solution to prepare stock solutions. Acetone control group was used in experiment and all experimental groups except the control group were made up to the same concentration of acetone which was 1 mL per liter.

Oregon stain (w.t.) third instar larvae of *D. melanogaster* were exposed to 0.1 mg/L, 1 mg/L and 10 mg/L BPA for six hours. During the BPA exposures, larvae were placed in glass tubes (2.5, 7.5 cm) containing drying papers that had absorbed stock solutions. Dose selection was based on results from our previous studies.

Observation of developmental stages

BPA exposed and non-exposed (control groups) larvae were placed in 250mL glass bottles that contained a standard *Drosophila* medium. The development of all experimental groups was observed at four-hour intervals by recording the number of individuals passing from larvae to pupae and from pupae to adults, and the transition periods. From the adults that emerged, virgin females were collected in order to use them in the "determination of the mean offspring number" experiment.

Determination of the mean offspring number

In order to determine the effects of the BPA on the daily mean offspring number, virgin females, hatched from the exposed larvae were used. An exposed female and 3 non-exposed males of same age were crossed. Parents were removed when the first pupa was seen. After the first adults began to hatch, the number of offspring emerging in 10 days were counted at 24-hr intervals.

Statistical Analysis

The statistical analysis of the results was carried out using the SPSS 11.5 programme. The determination of the significance of the transition percentages from larvae to pupae and from pupae to adults were obtained by an analysis of variance, and the comparison of the transition period from larvae to pupae and from pupae to adults were done by a two-variable t-test. The daily mean offspring number was calculated with the ANOVA test. For all statistical analysis, the criteria for significance was $p < 0.05$.

RESULTS

Effect of the BPA on the transition percentages from larvae to pupae

The pupated larvae from those exposed and non-exposed to the BPA were counted seperately and their transition percentages were determined (Table 1). Pupation percentages were found to be

Table 2. The changes of mean pupation time depending on BPA exposure doses.

Group No.	Groups	Mean Pupation Time (hour)	Significant Differences of the Means
1	Control	62.8	1-3* 1-4*
2	Acetone Control	63.1	1-5* 2-3*
3	0.1 mg/L (B1)	65.8	2-4* 2-5*
4	1 mg/L (B2)	66.0	
5	10 mg/L (B3)	65.3	

B: BPA exposure group; * : $p < 0.05$

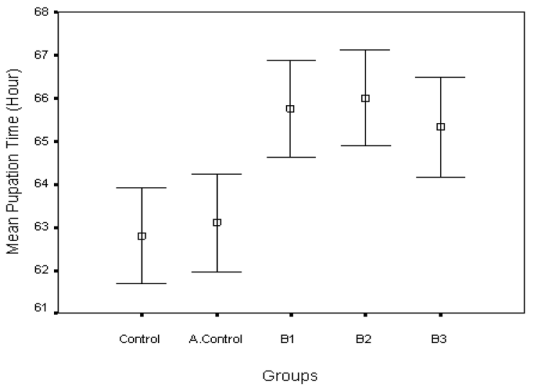

Figure 1. 95% confidence intervals of mean pupation time in the control (non-exposed) and the BPA exposed groups (B1, B2, B3).

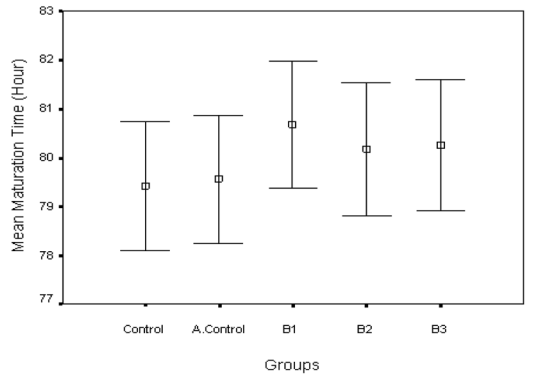

Figure 2. 95% confidence intervals of mean maturation time in the control (non-exposed) and the BPA exposed groups (B1, B2, B3).

96% in the control and the acetone control group, 98% in the 0.1mg/L BPA exposed group (B1), 95% in the 1mg/L BPA exposed group (B2), 92% in the 10mg/L BPA exposed group (B3). The result of a one variable variance analysis showed that BPA exposures did not have any effect in terms of the number of pupated larvae ($p > 0.05$).

Effects of the BPA on the mean pupation time

Table 2 shows the effect of different BPA doses on mean pupation time. The mean pupation time was 62.8 hours for the control group and 63.1 hours for the acetone control group. However, the mean pupation time increased to 65.8, 66 and 65.3 hours in the B1, B2 and B3 exposure group, respectively. This clearly shows that the mean pupation time was extended with BPA exposures in comparison to the control groups and that this increase was significant ($p < 0.05$). In another words, BPA exposures caused a developmental delay by extending the transition period from larvae to pupae. But no dose-response pattern was evident (Figure 1).

Effect of the BPA on the transition percentages from pupae to adult

The transition rates of BPA exposed and non-exposed flies from pupae to adult were determined and then compared statistically. As seen from Table 3, the adult formation rates were found to be 93.8% in the control and the acetone control group, 89.8% in the 0.1 mg/L BPA exposed group (B1), 85.3% in the 1 mg/L BPA exposed group (B2) and 92.4% in the 10 mg/L BPA exposed group (B3). A variance analysis showed that the differences between the groups were statistically insignificant at a level of $p > 0.05$.

Effects of the BPA on the mean maturation time

The mean maturation time was 79.4 hours for the control and the acetone control group. However, the mean maturation time increased to 80.7, 80.2 and 80.3 hours in the B1, B2 and B3 exposure group, respectively. It was found that the mean maturation time was delayed ($p < 0.05$) at concentrations ranging from 0.1 mg/L to 10 mg/L (Table 4), but no dose-response pattern was evident (Figure 2).

Table 3. The changes of the transition rate from pupae to adult depending on BPA exposure.

Groups	Number of pupae	Number of adult	Rate ± S.E.	S.D.	p
Control	96	90	93.8 ± 2.12	4.24	0.271
Acetone Control	96	90	93.8 ± 2.18	4.36	
0.1 mg/L (B1)	98	88	89.8 ± 1.32	2.63	
1 mg/L (B2)	95	81	85.3 ± 5.12	0.10	
10 mg/L (B3)	92	85	92.4 ± 3.15	6.29	

B: BPA exposure group, S.E.: Standard error, S.D.: Standard deviation, $p > 0.05$.

Table 4. The changes of mean maturation time depending on BPA exposure doses.

Group No.	Groups	Mean Pupation Time (hour)	Significant Differences of the Means
1	Control	79.4	1-3* 1-4*
2	Acetone Control	79.4	1-5* 2-3*
3	0.1 mg/L (B1)	80.7	2-4* 2-5*
4	1 mg/L (B2)	80.2	
5	10 mg/L (B3)	80.3	

B: BPA exposure group; * : $p < 0.05$

Effects of the BPA on daily mean offspring numbers

Table 5 shows the effect of different BPA exposure doses on the daily mean offspring number. The daily mean offspring number per female was 5.72 for the control group and 5.68 for the acetone control group. However, the daily mean offspring number was 4.85, 4.62 and 5.51 in the B1, B2 and B3 exposure group, respectively.

As seen in Table 5, there was a statistically significant reduction in the daily mean offspring numbers in the B1 and B2 compared to the control and the acetone control groups ($p < 0.05$). The other minor deviation, observed in the B3, was not significant statistically ($p > 0.05$).

DISCUSSION

BPA is one of the endocrine disrupting chemicals that, acting as xenoestrogens, interfere with the endocrine system in vertebrates through interaction with nuclear hormone receptors [2]. Moreover, it was found that BPA affects developmental and reproductive parameters in some invertebrates [1, 9, 11, 15]. In this study, the effects of BPA on the development and reproduction of *Drosophila melanogaster* (Diptera; *Drosophilidae*) were investigated. In our experiments, BPA exposures did not affect the pupation and maturation rates, but the mean pupation and maturation times were delayed. In addition, it was determined that there was a reduction in the daily mean offspring number of the 0.1 mg/L (B1) and 1 mg/L (B2) exposure groups compared to the control groups. This results suggest that BPA exposure can affected the development and reproduction of *Drosophila melanogaster*.

The most significant effect of BPA was the developmental delay in *D. melanogaster*. The delaying effect may be resulted from ecdysteroid hormone disruption. Ecdysteroids have essential roles in developmental changes in the life of *D. melanogaster* [16]. Ecdysteroids are steroid hormones. 20-hydroxyecdysone (20-E), one of the ecdysteroid, is biologically active form of ecdysone. 20-E exerts its effects on development through a heterodimeric complex of two nuclear receptor superfamily members; ecdysone receptor (EcR) and ultraspiracle (USP) [17, 18, 19]. This hormone trigger major developmental transitions in arthropods (*Drosophila*, *Chironomus* etc.). 20-E ecdysone receptor is the first target for the action of the hormone inside the cells. EcR is induced directly by ecdysone [2, 19].

Molecular studies have demostrated that the ecdysteroid receptor of *Drosophila* has homologies to the steroid hormone receptors of vertebrates in that the ecdysteroid receptor and other steroid receptors share a set of conserved features corresponding to the hormone and DNA-binding domain [20]. EDCs capable of binding to steroid hormone receptors can also bind to ecdysteroid receptors of invertebrates [1]. In a *Drosophila melanogaster* B_{II} cell in vitro assay, BPA was able to compete with ecdysteroids for the ligand binding site on the receptor complex [21].

Planello et al. [2] demonstrated that BPA significantly increases the mRNA level of EcR in

Table 5. Effect of BPA exposure on daily mean offspring number of D. melanogaster.

Group No.	Groups	Number of Female	Number of Offspring	Daily Mean Offspring Number ± S.E.	S.D.	Significant Differences of the Means
1	Control	64	3659	5.72 ± 0.24	6.07	
2	Acetone Control	54	3070	5.68 ± 0.24	5.50	1-3* 1-4*
3	0.1 mg/L (B1)	30	1457	4.85 ± 0.32	5.68	2-3* 2-4*
4	1 mg/L (B2)	33	1526	4.62 ± 0.30	5.40	
5	10 mg/L (B3)	32	1762	5.51 ± 0.35	6.35	

B: BPA exposure group, S.E.: Standard error, S.D.: Standard deviation.
* : $p < 0.05$

Chironomus riparius. This results demostrated that BPA really behaves as an ecdysone mimetic that is able to upregulate the levels of EcR. In our study, BPA might attached to ecdysteroid receptors and blocked them, preventing ecdysteroid hormones from binding to the receptor, thereby slowing the developmental processes.

It is known that developmental time is affected by many external and internal factors. For example, several studies have found that midly stressful developmental conditions, e.g. larval crowding, short term high temperature exposure and restricted diet, may lead to increased developmental time [22]. Lints and Lints [23] reported that changes in environmental factors cause a delay in the developmental phases, from the larval growth to death, by affecting the vital programmes and functions of molecules.

In the studies dealing with the effects of EDCs, it is emphasised that exposure to EDCs could induce stress. BPA have been shown to induce oxidative stress in the brain, liver, kidney, testis and epididymal sperm in rodents [24-29]. In order to protect itself, the cell repairs the proteins damaged due to stress or increases the production of special proteins. Some of these proteins are known as "heat shock proteins (hsps)" [30-32]. In a study done to determine the effects of stress factors such as heat, application of a stress factor (heat) caused hsp70 to increase and extended the developmental time. It was stated that the extension in the developmental time was a response to stress [22]. And in another study, it was shown that when the hsp70 transcription increased the delay in developmental time was occured [33].

Planello et al. [2] investigated the effect of 3-12 mg/L BPA exposure on the midge *Chironomus riparius* and found that BPA is able to increase hsp70 gene expession, which is commonly considered to be an indication of cellular stress. Similarly, it was found that the levels of hsp70 mRNA were increased by different pollutants including BPA [34]. The BPA that we applied might have spoilt the usual development by causing an increase in hsps expression.

During the larval-pupal metamorphosis, the expression of hsps is regulated by the steroid molting hormone ecdysone and there is a mutual interaction between the gene areas where they are coded [30]. Thus, hsps synthesis, induced under stress conditions, might have affected development by affecting the synthesis of ecdysone, which is an important hormone in development of Drosophila.

In the experiment to determine the daily mean offspring number of a female, it was observed that this number for the 0.1 mg/L (B1) and 1 mg/L (B2) exposure groups was less than that of the control groups ($p < 0.05$) (Table 5). The effects of EDCs on the number of offspring were investigated in previous works. Brennan et al. [35] searched the effects of 17 beta-oestradiol (E2), diethylstilbestrol (DES), bisphenol A (BPA) and 4-nonylphenol (4-NP) on the freshwater invertebrate *Daphnia magna*. They found

that no statistically significant ($p > 0.05$) inhibition in the number of offspring produced was observed when *D. magna* were exposed to E2 or BPA. But exposure of *D. magna* to 4-NP and DES decreased the number of offspring ($p < 0.05$). Similarly, Comber et al. [36] found that exposure to 4-NP resulted in significant ($p < 0.05$) reduction of the number of live offspring per adult *Daphnia* at concentrations of 0.056 mg/L, 0.10 mg/L and 0.18 mg/L. Baldwin et al. [37] reported that 0.05 mg/L and 0.1 mg/L 4-NP exposures were caused significant reduction in fecundity of *D. magna*. They subsequently postulated that, because of its phenolic structure, 4-NP might inhibit glucose and sulphate conjugation of steroids in invertebrates. These conjugated steroids are important elements in the steroid elimination/inactivation process in invertebrates and their disruption results in elevated hormone levels which adversely affect steroid hormone-dependent processes such as reproduction. BPA have a phenolic structure like 4-NP, thus similar effects might have occured with BPA exposures in our experiment.

In general, it is clear that BPA has effects upon development and reproduction. Based on the present report we conclude that BPA can cause developmental delay and decrease the number of offspring in *Drosophila melanogaster*. Invertebrates comprise approximately 95% of all terrestrial and aquatic animal species, so it is clearly necessary to determine the potential developmental and reproductive hazards posed by EDCs. It is well known that the effects of these chemicals on the development and reproduction of invertebrates are of great importance in the protection of the natural population's health. Therefore, more studies should be done to clarify the effect mechanisms.

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