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Abstract Bisphenol A (BPA), 4-nonylphenol (4-NP) and 4-*tert*-octylphenol (4-*tert*-OP) are the endocrine disrupting chemicals (EDCs) that has been shown to exert both toxic and biological effects on living organisms. The present study investigated effects of environmentally relevant concentrations of BPA, 4-NP and 4-*tert*-OP (0.1, 1 and 10 mg/L) on the fecundity of fruit fly *Drosophila melanogaster*. In the all exposure groups of BPA, 4-NP and 4-*tert*-OP, it was found a statistically significant decrease in mean fecundity as compared to the control groups ($p < 0.05$).

Keywords *Drosophila melanogaster* · Bisphenol A · 4-nonylphenol · 4-*tert*-octylphenol · Fecundity

Endocrine disrupting chemicals (EDCs) have recently received considerable attention. Many studies have shown that EDCs have the potential to modulate or disrupt the synthesis, secretion, transport, binding, action or elimination of endogenous hormones in the body and consequently to affect homeostasis, development, reproduction and behavior of organisms (Segner et al. 2003). Bisphenol A (BPA), 4-nonylphenol (4-NP) and 4-*tert*-octylphenol (4-*tert*-OP) belong to the industrial chemicals that have received considerable attention due to high production and widespread usage. BPA is a monomer utilized to manufacture polycarbonate plastic and epoxy resins. The potential exposure of human is high because it is widely used in baby bottles, as protective coatings on food cans, as well as for dental sealants ad composites (Sonnenschein and Soto 1998; Xu et al. 2005). 4-NP and 4-*tert*-OP are used in a wide variety of industrial aplications, such as paper and textile industries, agricultural pesticides, water-based paints, ink, domestic and industrial cleaning substances, toys, contact lenses, spermicides in condoms, among others (Cakal Arslan and Parlak 2007).

Studies on the effects of EDCs have centered mainly on effects in vertebrates. Despite the fact that invertebrates represent more than 95 % of the known species in the animal kingdom, more detailed information about the effects on and mechanisms of action in invertebrates has only been obtained from a few cases (deFur et al. 1999; Lemos et al. 2010). The limited number of examples for endocrine disruption in invertebrates is partially due to the fact that their hormonal systems are rather poorly understood in comparison with vertebrates. Deleterious endocrine changes following an exposure to certain compounds may therefore easily be missed or simply be unmeasurable at present, even though a number of field investigations and laboratory studies show that endocrine disruption as probably occurred (deFur et al. 1999; Oehlmann et al. 2000).

In this study, the effects of BPA, NP and OP on the fecundity of *Drosophila melanogaster* was examined. Fruit fly *D. melanogaster*, a dipterian insect, due to its short life span well defined genetics, easy to rear in laboratory, offers many advantages for the detection of mutagenic, morphological and developmental effect of different chemical agents. It provides the quickest assay system for detecting adverse effects of EDCs. The present study is the first to examine the effects of BPA, 4-NP and 4-*tert*-OP, EDCs used in the manufacture of many industrial products, on the fecundity of the fruit fly.

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Materials and Methods

In this study, the wild type Oregon strain of *D. melanogaster* was used. The flies were kept in a *Drosophila* culture room (Hacettepe University, Ankara, Turkey) at 25°C and relative humidity of 50 %–60 % and in 12 h light, 12 h 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 h. 72 ± 4 h later, the third instar larvae were collected.

The solutions of BPA, 4-NP and 4-*tert*-OP were prepared from solid compounds (Sigma-Aldrich; Steinheim, Germany). A known amount of BPA, 4-NP and 4-*tert*-OP 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, 1 and 10 mg/L BPA, 4-NP and 4-*tert*-OP for 6 h. During the exposures, larvae were placed in glass tubes (2,5 × 7,5 cm) containing drying papers that had absorbed stock solutions. Thus, larvae were exposed these chemicals by nutrition as well as absorption through the skin. Dose selection was based on results from our previous studies (Atlı and Ünlü 2008, 2009). These doses were environmentally relevant concentrations. In additon, they did not cause any lethal effect on *D. melanogaster* larvae, so reproductive effects of these chemicals were determined.

In order to determine the effects of BPA, 4-NP and 4-*tert*-OP on the mean fecundity, virgin females developed from exposed larvae were used. An exposed female and 3 non-exposed males of same age (3 days old) were crossed in empty glass culture bottles. Then spoons, containing standard medium, were placed in these culture bottles immediatelly. These spoons were changed for every 24 h and the eggs were counted for a period of 10 days. It was stated that the egg production in the first 10 days of adult life was a good reference for the whole adult life egg production of this organism (Yesilada 1999; Atlı and Ünlü 2007). The statistical analysis of the results was carried out using the SPSS 11.5 programme. The daily mean egg production in each group was calculated with the ANOVA test. A Games–Howell multiple comparison test versus control groups was used.

Results and Discussion

The effects of the three test compounds (BPA, 4-NP and 4-*tert*-OP) on the fecundity of *D. melanogaster* were investigated 10 days period. The results of these tests are detailed in Tables 1, 2 and 3. The daily mean egg-production per female during the first 10 days of adult life was 6.85 and 7.08 for non-exposed control groups. As seen in Tables 1, 2 and 3, there was a statistically significant reduction in the daily mean egg productions in the all doses of BPA, 4-NP and 4-*tert*-OP exposure groups ($p < 0.05$) compared to the control group. Under the experimental conditions, the factors that might have affected the egg production were kept stable. Thus, the differences in the results were interpreted as being caused by the chemicals exposed.

In publications dealing with the effects of these three EDCs, specific reproductive effects of exposure have not been clearly shown, and the effects have not always occurred in the same direction. While in some publications it was reported that BPA, 4-NP and 4-*tert*-OP decreased fecundity (Comber et al. 1993; Baldwin et al. 1997; Shurin and Dodson 1997; Preston et al. 2000; Bettinetti and Provini 2002; Fukuhori et al. 2005; Mihaich et al. 2009), in others it was reported that they increased fecundity (Marcial et al. 2003; Widarto et al. 2007) or not caused any reproductive effect (Kahl et al. 1997; Forget-Leray et al. 2005; Forbes et al. 2008).

Comber et al. (1993) found that first generation exposure to 4-NP resulted in significant ($p < 0.05$) inhibition of the number of live offspring per adult *Daphnia magna* at concentrations of 0.056, 0.10 and 0.18 mg/L. Baldwin et al. (1997) reported significant reduction in fecundity of first generation *D. magna* following exposure to 0.05 and 0.1 mg/L. Similarly, prenatal exposure to NP has resulted in reduced fecundity in adult female *Daphnia* (Shurin and Dodson 1997). In another work, it was reported that nonylphenol caused a reduction in fertilization and resting egg production in the freshwater rotifer *Brachionus calyciflorus* at concentrations in the 1–50 µg/L range (Preston et al. 2000). In another study, twenty-eight-day tests were performed to evaluate the toxicity and the effects on reproduction of 4NP spiked sediment to the benthic invertebrates *Tubifex tubifex* and *Chironomus riparius*. No significant differences were noted in the *sex ratio* of the emerged chironomids when exposed to 4NP compared to the controls, but the emerged chironomids did not lay eggs at concentrations higher than the EC_{10} (250 µg 4NP g^{-1} dw) (Bettinetti and Provini 2002). Fukuhori et al. (2005) investigated the toxic effects of BPA on the sexual and asexual reproduction of *Hydra oligactis*, an evolutionarily primitive invertebrate. It was found that the mean number of eggs per polyp significantly decreased as BPA concentration increased from 2 mg/L to 4 mg/L ($p < 0.05$). Mihaich et al. (2009) found that 1.5 mg/L BPA exposure was caused statistically significant reduction in fecundity of female amphipod *Hyalella azteca*. These studies show

Table 1 The effect of BPA exposure on daily mean egg production of *D. melanogaster*

Group no	Groups	No. of female	No. of egg	Daily mean egg production ± SEM	SD	Significant differences of the means
1	Control	25	1,712	6.85 ± 0.52	8.147	
2	Acetone control	25	1,770	7.08 ± 0.52	8.163	1–3* 2–3*
3	0.1 mg/L (B1)	25	1,068	4.27 ± 0.25	4.007	1–4* 2–4*
4	1 mg/L (B2)	25	1,093	4.37 ± 0.27	4.344	1–5* 2–5*
5	10 mg/L (B3)	25	1,175	4.70 ± 0.31	4.861	

B bisphenol A exposure group, *SEM* standard error of mean, *SD* standard deviation

* $p < 0.05$

Table 2 The effect of 4-NP exposure on daily mean egg production of *D. melanogaster*

Group no	Groups	No. of female	No. of egg	Daily mean egg production ± SEM	SD	Significant differences of the means
1	Control	25	1,712	6.85 ± 0.52	8.147	
2	Acetone control	25	1,770	7.08 ± 0.52	8.163	1–3* 2–3*
3	0.1 mg/L (N1)	25	929	3.72 ± 0.19	3.050	1–4* 2–4*
4	1 mg/L (N2)	25	887	3.55 ± 0.19	3.069	1–5* 2–5*
5	10 mg/L (N3)	25	931	3.72 ± 0.19	3.055	

N nonylphenol exposure group, *SEM* standard error of mean, *SD* standard deviation

* $p < 0.05$

Table 3 The effect of 4-*tert*-OP exposure on daily mean egg production of *D. melanogaster*

Group no	Groups	No. of female	No. of egg	Daily mean egg production ± SEM	SD	Significant differences of the means
1	Control	25	1,712	6.85 ± 0.52	8.147	
2	Acetone control	25	1,770	7.08 ± 0.52	8.163	1–3* 2–3*
3	0.1 mg/L (O1)	25	848	3.39 ± 0.19	3.000	1–4* 2–4*
4	1 mg/L (O2)	25	846	3.38 ± 0.21	3.384	1–5* 2–5*
5	10 mg/L (O3)	25	865	3.46 ± 0.18	2.813	

O octylphenol exposure group, *SEM* standard error of mean, *SD* standard deviation

* $p < 0.05$

that EDCs may cause a decreasing effect upon fecundity, and support our findings.

Many external (temperature, humidity, nutrition, population density etc.) and internal factors (genetic structure, age etc.) affect fecundity of *D. melanogaster* (Ashburner 1989). It was reported that there was considerable changes in the *Drosophila* reproductive functions under external stress factors and that ecdysteroid hormones (ecdysone and 20-hydroxyecdysone) was involved these events. Both males and females have a certain base level of ecdysone, which is known to be a prohormone convertible into a hormone, 20-hydroxyecdysone, in the target tissues. As 20-hydroxyecdysone controls the ovary development it is found in females higher than males. It was reported that there is negative feedback mechanism between 20-hydroxyecdysone quantity and egg production. Therefore, any effect for increasing the quantity of 20-hydroxyecdysone will reduce the egg production (Rauschenbach et al. 2000). The results of the some studies demostrated that EDCs induced cellular stress by caused hydroxy radical formation (Roy et al. 1997; Obata and Kubota 2000). In our study, exposure of three selected EDCs caused a statitically significant reduction in the egg production of *D. melanogaster*. The reason of this reduction may be the increased proportion of ecdysteroid hormones because of stress conditions caused by EDCs.

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)'' (Krebs and Feder 1998; Morrow and Tanguay 2003). Basically, most of the hsps are involved in folding and assembly of native proteins. However, under stress conditions, hsps migrate into the cell nucleus where they act to repair or protect the nuclear proteins and minimize protein aggregation preventing genetic damage (Rhee et al. 2009). Induction of hsp70 in response to exposure to EDCs has been reported in some invertebrates (Pyza et al. 1997; Snyder and Mulder 2001; Rhee et al. 2009). Conditions known to induce hsp synthesis reduce fecundity in female *Drosophila* (Krebs and Loeschcke 1994). A significant reduction in fecundity at all concentrations of BPA, 4-NP and 4-*tert*-OP in the present study may be attributed to induced hsp synthesis with exposed chemicals.

Risk from the release of EDCs into the environment as a result of their use for various purposes requires assessment to evaluate the probable adverse effects to occupationally exposed population. With the availability of genome sequence data for various organisms, and with the invention of advanced bioinformatics tools, it is now clear that genes *Drosophila* share strong homology with majority of the genes of higher animals. For example, about 75 % of known human disease genes have a recognizable match in the genome of fruit flies, and 50 % of fly protein sequences have mammalian homologs (Reiter et al. 2001). Therefore, our investigations using *Drosophila* as an alternate animal model offers a unique opportunity to extrapolate the exposure impact of EDCs to higher animals quickly and sensitively.

In conclusion, the present study suggests that BPA, 4-NP and 4-*tert*-OP have effects on fecundity. Based on the present report we conclude that these three selected EDCs cause a reduction in the number of egg production in *D. melanogaster*. Studies on the effects of EDCs have centered mainly on effects in vertebrates. From many studies reported in the literature about endocrine disruption, only a minor fraction have investigated their effects in invertebrates; from these only 10 % were conducted with terrestrial invertebrates (Lemos et al. 2010). 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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