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Effects of bisphenol A and ethynyl estradiol exposure on enzyme activities, growth and development in the fourth instar larvae of *Chironomus riparius* (Diptera, Chironomidae)

Se-Bum Lee, Jinhee Choi*

Faculty of Environmental Engineering, College of Urban Science, University of Seoul, 90 Jeonnong-Dong, Dongdaemun-gu, Seoul 130-743, Korea

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Abstract

This study was conducted to determine the toxic effects of bisphenol A (BPA) and ethynyl estradiol (EE), well-known endocrine disruptors, on *Chironomus riparius* under controlled laboratory conditions. Mortality, enzyme activities, and growth/development parameters were studied as acute, biochemical, and physiological toxicities, respectively. The results of the present study showed activation of catalase and glutathione-*S*-transferase after BPA and EE exposure, as well as increased emergence failure after EE exposure. This study on the effects of BPA and EE on *C. riparius* can be an important addition to the knowledge that has been obtained regarding the toxicology of BPA and EE in aquatic organism, on which limited data are available. The data obtained from this study, however, are not sufficient to establish any correlation or casual relationship between these two compounds and the response of *C. riparius*. Thus, further research is required to come up with direct experimental demonstrations of the wider relationship between the biochemical effects of BPA and EE on *C. riparius* and their consequences at higher levels of biological organization.

Keywords: *Chironomus riparius*; Bisphenol A; Ethynyl estradiol; Sublethal toxicity; Biomarker

1. Introduction

The water contaminants bisphenol A (BPA) and ethynyl estradiol (EE), well-known endocrine-disrupting chemicals (EDCs) frequently found in freshwater, have recently attracted attention. An industrial chemical, BPA is an intermediate in the production of polycarbonate and epoxyresins (Staples et al., 1998), and a pharmaceutical, EE is a synthetic estrogen used as a female contraceptive (Purdom et al., 1994). They are known to elicit estrogenic responses in fish via interaction with the cellular receptor, and have been reported to be present in surface waters at concentrations well beyond those known to cause endocrine disruption (Larsson et al., 1999). In recent years, the association of altered hormonal regulation in humans and wildlife with their exposure to EDCs has led to increasing public and scientific concern (Colborn et al., 1996).

Despite the importance of BPA and EE toxicity in the aquatic ecosystem, only a few studies have been conducted on the effects of these compounds on the components of the aquatic ecosystem. The aquatic larvae of non-biting midges (Chironomidae, Diptera) are globally distributed, and these non-biting midges are the insects most often found in freshwater ecosystems. They play an important role in the aquatic food chain and are a major food source of fish and other vertebrates, as well as invertebrates (Cranston, 1995). They are easy to culture, are sensitive to many pollutants, and have a short life cycle (Ingersoll and Nelson, 1990). As such, they are widely used in ecotoxicity tests (Bettinetti et al., 2002; Choi et al., 2000, 2002; Crane et al., 2002; Kahl et al., 1997; Matthew and David, 1998; Matthew et al., 2001). Taking into account the frequent presence of BPA and EE in surface waters and the ecological importance of *Chironomus* larvae in freshwater,

*Corresponding author. Fax: +82 2 2244 2245.
E-mail address: jinhchoi@uos.ac.kr (J. Choi).

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this study on the effects of BPA and EE on *Chironomus riparius* can provide information that may prove to be valuable for the biomonitoring or risk assessment of the aquatic ecosystem.

In this study, median lethal concentration (LC50) was determined as the acute toxicity indicator of BPA and EE on *C. riparius*, and the biochemical/physiological parameters of *C. riparius* were investigated after their sublethal exposure to BPA and EE. BPA and EE concentrations were measured in test water by enzyme-linked immunosorbent assay (ELISA). Among the biochemical parameters, the chemical effects of the two compounds on enzyme activities were focused on. Catalase (CAT), peroxidase (Px), and glutathione peroxidase (GPx) were studied as oxidative stress markers. Glutathione-*S*-transferase (GST) and acetylcholine esterase (AChE) were measured as detoxification and neurotoxicity indicators, respectively. As for the physiological responses, growth and development were studied. The dry-body weights of the larvae were measured, as the growth indicators, whereas successes in pupation and in adult emergence as well as the total emergence times were examined as descriptors of development. The adult sex ratio was also studied to identify any potential difference between male and female susceptibility to these compounds.

2. Materials and methods

2.1. Organisms

Using an original strain provided by the Toxicology Research Center of the Korea Research Institute of Chemical Technology (Daejeon, Korea), *C. riparius* larvae were obtained from adults reared in the laboratory. The larvae, which were fed Tetramin®, were reared within a 16–8 h light–dark photoperiod, at room temperature (20±1 °C), in a 2 L glass chamber containing dechlorinated tap water and aerated acid-washed sand.

2.2. Exposure conditions

The effects of BPA and EE exposure on the groups of the fourth instar larvae collected from the rearing aquaria were assessed. At the beginning of the experiment, 1 mL of an acetonic solution of the compounds was placed in the experimental 1 L tanks. Fifty larvae (of sublethal toxicity) were then randomly introduced into each test aquarium. The exposure was carried out at a constant temperature (20±1 °C) and within a photoperiod of 16:8 h (light:dark) in all the experiments. The enzyme activities and growth indicators in six sublethal concentrations (0.001, 0.01, 0.1, 1, 10, and 100 μg/L) of the compounds were studied. The emergence in three higher concentrations (0.1, 1, and 10 μg/L) of both compounds was also studied.

2.3. Detection of BPA and EE by ELISA

Concentrations of BPA and EE in water were determined using a BPA ELISA Kit and a 17β-Estradiol ELISA Kit (Japan EnviroChemicals Ltd., Tokyo, Japan), respectively. Ten μg/L of BPA and 1 μg/L of EE were spiked into water. At 12, 24, 48, 72, and 96 h after water spiking, 1 mL of water was collected in experimental tubes. Water was filtered through paper filters and introduced into a glass vial and pre-conditioned with 10% methanol and mixed with the enzyme-labeled BPA and EE solutions, respectively and 0.1 mL transferred to separate wells of a 96-well plate coated with anti-BPA and anti-EE antibodies, respectively. The plate was then incubated at room temperature for 60 min. Unbound antibodies were removed by washing three times with the wash buffer. Then 0.1 mL per well of color solution was added and the plate was incubated for 30 min, after which the reaction was inhibited by the addition of stop solution. The absorbance was then measured at 450 nm using a Micro-Plate Reader (Tecan, Mannedorf, Switzerland).

2.4. Acute toxicity test

A group of 10 larvae was exposed to four concentrations of BPA and EE, and the others were made control groups. It was determined that acute toxicity occurs after 24 h exposure, and that the condition eventually leads to death. Log-probit data transformation was used to estimate the 24 h LC50 values and the corresponding 95% confidence intervals.

2.5. Enzyme activities

A total of 10 larvae were collected from the control and experimental tanks 24 h after these were treated with the compound solution. The larvae were then pooled for enzyme activity measurements. They were homogenized in 2.5 mL Tris-EDTA buffer (40 mM, pH 7.8; Sigma-Aldrich, Saint Quentin Fallavier, France) with the use of a Potter-Elvehjem homogenizer. Crude homogenate was centrifuged for 15 min at 500*g* (4 °C), and supernatant was centrifuged for 30 min at 12,000*g* (4 °C). The resulting supernatant (a post-mitochondrial fraction) was used to measure the enzyme activities. The rate of H_2O_2 disappearance (measured at 240 nm) was used to quantify the CAT activity (Beers and Sizer, 1952). The total Px activity was measured through a guaiacol test (George, 1953). The method described by Paglia and Valentine (1967) was employed to determine the GSH-Px activity, whereas the GST activity was assessed spectrophotometrically through the measurement of glutathione-1-chloro-2,4-dinitrobenzene (CDNB) conjugate production (Habig et al., 1974). The AChE activity was measured with the use of the method introduced by Ellman et al. (1961). The enzyme activities were calculated relative to the protein contents of the extracts, which were measured with the use of the Bradford method (Bradford, 1976).

2.6. Body fresh/dry weight measurement

For the measurement of body fresh/dry weights, 10 larvae collected after 48 h of exposure to the compounds. The fresh weights were immediately measured. The dry-body weights of the larvae were also measured after they were exposed to a temperature of 105 °C for 24 h, and the water contents were calculated from the respective dry and fresh weights of the larvae. The weights were rounded off to the nearest 0.1 mg.

2.7. Adult emergence rate

For the measurement of the adult emergence rate, 50 of the fourth instar larvae were introduced at the beginning of the experiment. The emerging adults were retained with the use of wood cages covered with steel-wire mesh until the emergence had been completed in the control and experimental aquaria. As the endpoints of the toxicity tests, the numbers of the emerged adults from each vessel were counted and their sexes were determined. The two sexes could be easily distinguished from each other based on the forms and lengths of their antennae and abdominal terminalia. In addition, the dead pupae were counted for estimating pupation failure rate. Every 2 days, 50 mg of Tetramin fishfood flakes was supplied to each aquarium. The test solutions were not renewed. All the data were recorded at daily intervals.

2.8. Chemicals

BPA and EE were purchased from Sigma (Sigma Corp., St. Louis, MO, USA).

2.9. Data analysis

Statistical differences between the control and treated larvae were examined with the aid of a parametric *t* test using SPSS 12.0KO (SPSS Inc., Chicago, IL, USA). An alpha level of 0.05 was used to determine significance in all statistical analyses.

3. Results

BPA and EE residues were detected in water by ELISA, during 96 h (Fig. 1). The nominal concentration of BPA and EE in test water at the beginning of the kinetics experiment was 10 and 1 μg/L, respectively. Results of the 96 h-long study show that BPA concentration decreased rapidly during the first 24 h and that less than 10% of the initial amount remained after 24 h and it maintained until the end of the experiment. Concentration of EE in water decreased more slowly than that of BPA. After 72 h, the concentration in water was reached about 20% of the initial amount and it maintained until the end of the experiment.

The LC50s of BPA and EE, to which the fourth instar larvae of *C. riparius* were exposed for 24 h, were estimated (Table 1). The LC50s of BPA and EE in *C. riparius* larvae were 6.03 and 9.14 mg/L, respectively. Based on the results of the acute-toxicity test, six concentrations corresponding to 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} of the 24 h LC50 (0.01, 0.01, 0.1, 1, 10, and 100 μg/L) were selected for sublethal exposure levels. A development study was then conducted on the three highest concentrations (1, 10, and 100 μg/L), since alteration of this parameter was hardly expected at lower exposure levels.

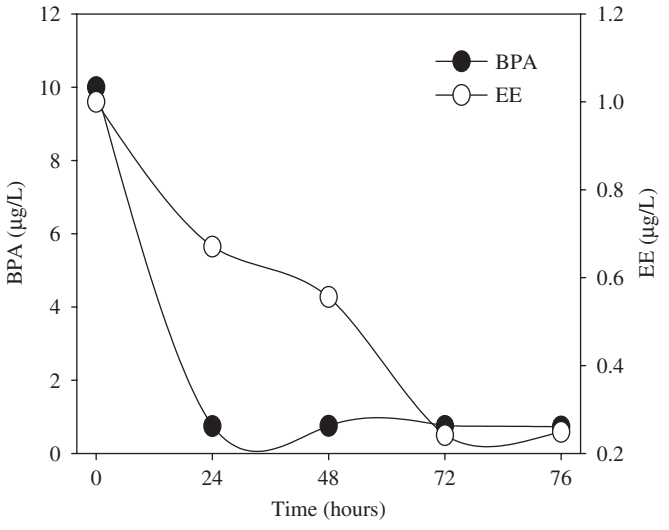


Fig. 1. Concentrations of BPA and EE in water measured by enzyme-linked immunosorbent assay (ELISA).

Table 1
Estimation of 24 h LC50 of BPA and EE in fourth instar larvae of *C. riparius*

	24 h LC50 (mg/L)	95% Confidence interval
BPA	6.030	3.788 < LC50 < 7.410
EE	9.136	7.341 < LC90 < 24.97

As for the biochemical parameters, the enzyme activities in the fourth instar larvae of *C. riparius* that were exposed to BPA or EE for 24 h were measured (Table 2). The CAT activity increased at 0.001 and 0.01 μg/L of BPA, and increased at all the concentrations of EE. The Px activity decreased at 0.1 and 10 μg/L of BPA exposure and increased at 0.1, 10, and 100 μg/L of EE exposure. No significant change was observed in the GPx and AChE activities. The GST activity increased after exposure to 0.01, 1, and 10 μg/L of BPA and after exposure to 0.1, 10, and 100 μg/L of EE.

As growth indicators, the dry body weights and fresh body weights of the *C. riparius* larvae were measured after a 48 h exposure to BPA or EE (Table 3). An increase in dry body weights was observed after exposure to the lower concentrations (0.001, 0.01, and 0.1 μg/L) of BPA and to the higher concentrations (1, 10, and 100 μg/L) of EE. No significant change was observed in fresh body weights, except after exposure to 0.001 μg/L of BPA.

As development parameter, BPA and EE-induced pupation and emergence failure was studied in *C. riparius* (Table 4). Development was not affected by BPA exposure. EE exposure appeared to have no significant effect on pupation, whereas it induced emergence failure at the high concentration (100 μg/L of EE). The degree of failure was more significant when counted from the larva to adult than when counted from the pupa to adult.

Effects of BPA and EE on the kinetics of emergence in *C. riparius* were studied via cumulative method (Figs 2 and 3). Total adult emergence rate decreased by BPA (10 μg/L, Fig. 2A) and EE exposure (100 μg/L, Fig. 3A). However, no significant difference was found from group to group in male and female emergence rates.

Table 5 shows alteration in sex ratios induced by BPA and EE. BPA exposure seemed to induce slight male adult rate, whereas female adult rate slightly increased by EE exposure. Increased male/female ratio after exposure to 1 μg/L BPA was the most significant.

4. Discussion

This study was designed as a short-term experiment under controlled laboratory conditions, using actual measured concentration of BPA and EE, to demonstrate toxic effects of these compounds on enzyme activities, growth and development of *C. riparius*. Taking into account the ecological importance of *Chironomus* in the aquatic ecosystem, this study on the effects of BPA and EE, emerging water contaminants, on *C. riparius* can

Table 2
Enzyme activities measured in the fourth instar larvae of *C. riparius* exposed to sublethal concentration of BPA and EE for 24 h ($n = 3$, mean ± standard error of mean, $^{*}P < 0.05$)

Enzymes	CAT (U/mg protein)		PX (U/mg protein)		GPX (U/mg protein)		GST (U/mg protein)		AChE (U/mg protein)	
Exposure (μg/L)	BPA	EE	BPA	EE	BPA	EE	BPA	EE	BPA	EE
0 (control)	0.46±0.04		6.60±0.58		0.43±0.09		0.10±0.00		0.38±0.06	
0.001	0.74±0.07*	0.73±0.05*	5.20±0.80	6.60±1.60	0.27±0.03	0.19±0.07	0.27±0.06	0.13±0.02	0.46±0.04	0.52±0.07
0.01	0.86±0.06*	1.13±0.18*	6.50±1.50	6.90±1.20	0.19±0.04	0.18±0.16	0.16±0.01*	0.12±0.03	0.26±0.03	0.35±0.03
0.1	0.60±0.10	0.92±0.07*	4.40±0.20*	12.0±1.40*	0.30±0.03	0.25±0.05	0.20±0.05	0.22±0.02*	0.40±0.04	0.46±0.06
1	0.52±0.04	1.09±0.19*	8.00±0.50	13.0±2.50	0.31±0.04	0.50±0.15	0.15±0.01*	0.13±0.02	0.36±0.05	0.37±0.06
10	0.63±0.09	0.80±0.05*	3.80±0.10*	11.0±0.10*	0.35±0.001	0.17±0.04	0.19±0.00*	0.19±0.02*	0.40±0.05	0.44±0.06
100	0.47±0.63	0.89±0.08*	5.90±0.60	11.0±1.20*	0.15±0.01	0.76±0.09	0.18±0.05	0.21±0.02*	0.41±0.06	0.46±0.03

Table 3
Dry and fresh body weights measured in the fourth instar larvae of *C. riparius* exposed to sublethal concentration of BPA and EE for 24 h ($n = 3$, mean ± standard error of mean, $^{*}P < 0.05$)

Exposure (μg/L)	Body dry weight (mg/larvae)		Body fresh weight (mg/larvae)	
	BPA	EE	BPA	EE
0 (control)	0.53±0.01		4.21±0.16	
0.001	0.71±0.03*	0.54±0.06	4.99±0.99*	4.09±0.43
0.01	0.73±0.04*	0.61±0.04	4.79±0.18	4.48±0.29
0.1	0.75±0.01*	0.52±0.09	4.9±0.03*	4.2±0.33
1	0.75±0.09	0.75±0.03*	5.12±0.39	4.93±0.27
10	0.64±0.05	0.62±0.02*	4.52±0.29	4.58±0.19
100	0.63±0.05	0.69±0.01*	4.42±0.13	4.68±0.08

Table 4
Pupation and adult emergence failure rate measured in sublethal concentration of BPA and EE-exposed *C. riparius* (means ± standard error of mean, $^{*}P < 0.05$)

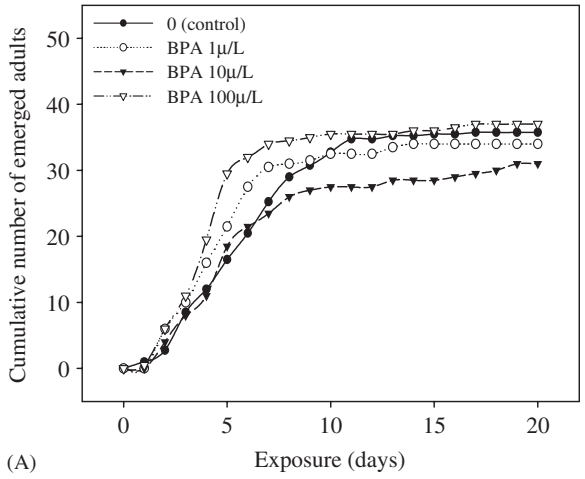
Exposure (μg/L)	Pupation failure (%)		Emergence failure (%)			
			From pupa		From larva	
	BPA	EE	BPA	EE	BPA	EE
0 (control)	14±10		15±1		29±11	
1	20±6	20±10	12±2	12±6	32±4	32±16
10	28±8	17±1	12±0	13±5	38±8	30±6
100	12±4	16±2	14±2	24±2*	26±6	40±4*

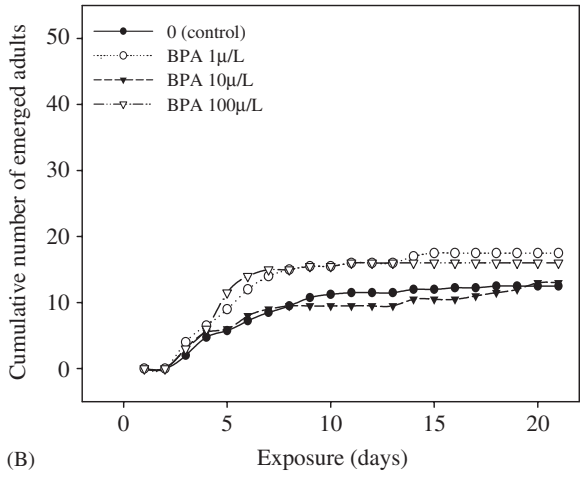
provide information that may prove to be useful in determining the potential ecological consequences of these compounds.

For quantitative determination of EDCs, gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry/mass-spectrometry are generally employed. While these methods can be reliable, they have several drawbacks including expensive instrument, large sample volume, extensive purification, technical expertise in operation, and lengthy analysis. Therefore, rapid, simple, and cost-effective methods, such as ELISA, were developed and recently widely used for quantification of EDCs. Even though, ELISA systems have drawbacks that may give the overestimated value because of the cross-reactivity, ELISA have significant advantage over more traditional analytical methods for environmental monitoring; they are quick, inexpensive, simple to perform, and can be highly sensitive (Goda et al., 2000). The kinetics study of BPA and EE concentrations by ELISA method revealed a rapid degradation of these compounds in test water, especially BPA (Fig. 1). From this result and considering the relatively high polarity of these compounds, long-term bioaccumulation of these compounds in *Chironomus* is hardly expected. However, if BPA and EE concentrations in *Chironomus* (internal dose) had also been measured, this could probably be better explained. Despite its associated difficulties and limitations, the use of the measured concentration of chemical compounds may have distinct advantages over using nominal concentrations as a toxicological index. In terms of toxicity, chemical fate data in the environment, where the test organism exposed, can be explained more meaningfully, and make a better connection between the accumulated dose and the toxicological effect, thus permitting better interpretation of the hazard associated with complex exposure routes.

Chironomus seems to have an efficient biochemical defense mechanism, which may contribute to the organism's tolerance of various environmental stresses, including chemical pollutions. Previous studies have shown that enzymatic radical scavengers, including superoxide dismutase (SOD), CAT, Px, and GPx could be developed as non-stressor-specific biomarkers in *C. riparius* larvae (Choi et al., 1999, 2000). Various molecular/biochemical parameters measured in *C. riparius* larvae, such as heat shock protein and hemoglobin genes expression, AChE, SOD, GST, electron transport system, and energy-yielding substrates, have already demonstrated a high sensitivity to environmental pollutants (Choi et al., 2000, 2001; Olsen et al., 2001; Lee and Choi, 2006; Lee et al., 2006).

The enzyme activities were investigated to identify suitable biomarkers for screening the biochemical level response of chemical exposure and thus applicable for in situ ecotoxicity monitoring. Enzymes selected in this study





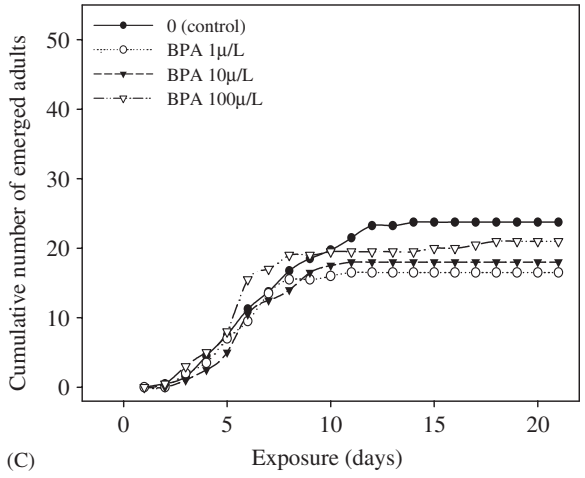
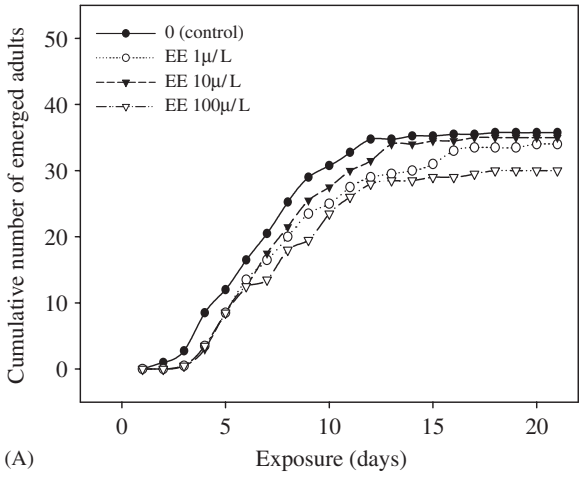
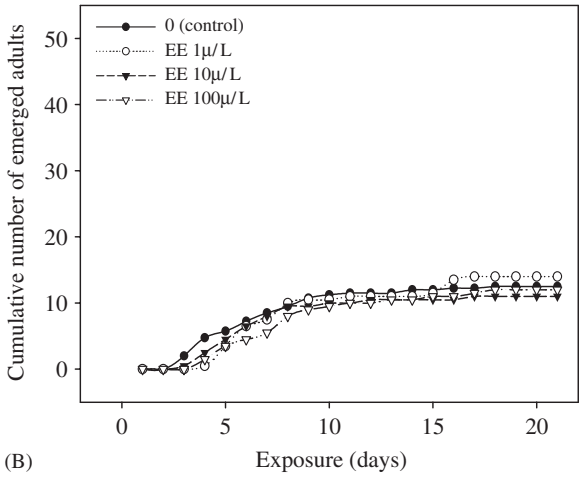


Fig. 2. Cumulative emergence of total (A), male (B), and female adults (C) measured in BPA-exposed *C. riparius.* Results are expressed as percentage of the total number of larvae introduced at the beginning of the experiment.





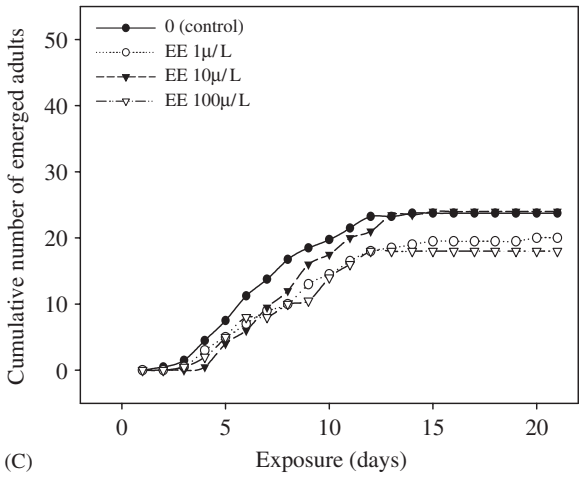


Fig. 3. Cumulative emergence of total (A), male (B), and female adults (C) measured in EE-exposed *C. riparius.* Results are expressed as percentage of the total number of larvae introduced at the beginning of the experiment.

meet this purpose, which are easy to measure and have broad range of cellular functions, including oxidative stress, detoxification, and neurotoxicities. Among five enzymes tested, the CAT activities seemed the most sensitive in both compounds. The early response of CAT to low concentration of BPA and EE (0.001 μg/L) may be considered a homeostasis-maintaining process rather than an indicator of the permanent adverse effects of these compounds. The homeostatic responses of CAT seem to have little impact at higher levels of biological organiza-

Table 5
Male and female emergence rate in sublethal concentration of BPA and EE-exposed *C. riparius*. (means ± standard error of mean)

Exposure (μg/L)	Male (%)		Female (%)	
	BPA	EE	BPA	EE
0 (control)	38.1 ± 9.70		61.9 ± 9.70	
1	50.9 ± 10.2	39.9 ± 5.30	49.1 ± 10.2	60.1 ± 5.30
10	41.4 ± 4.30	31.2 ± 3.00	58.6 ± 4.30	68.8 ± 3.00
100	41.8 ± 18.2	33.4 ± 9.90	58.2 ± 18.2	66.6 ± 9.90

tion. An increase in CAT at high EE concentrations (>1 μg/L) occurred concomitantly with Px activation, which may reflect an adverse effect of the exposure, since perturbations of the physiological parameters (i.e., increase in emergence failure) were observed at these concentrations. Peroxidasic activity is a well-known property of hemoglobin (Everse et al., 1990). Our previous study shows that under normoxic conditions, hemolymph contains about 90% of total Px activity and that hemoglobin content and Px activity are positively correlated (Choi et al., 2000). Thus, altered Px activity by BPA and EE might be essentially hemoglobin-dependent. However, our experiment only deals with some antioxidant enzyme activities, which are not sufficient to provide a clear explanation for the described phenomenon. If related oxidative stress parameters (i.e., measurement of free radicals, non-enzymatic antioxidant levels, such as, GSH and oxidative DNA or lipid damages) had concomitantly been investigated, this could probably be evaluated and explained to a greater extent.

The increased GST activity after BPA and EE exposure can be attributed to the possible involvement of the GSH conjugation pathway in the detoxification/metabolism of these compounds. The AChE activity did not change after BPA and EE exposure, which suggests that neurotoxicity may not be important in the toxicity of these compounds in *C. riparius* larvae. The LC50s of BPA and EE on *C. riparius* were determined to be around 6–9 mg/L, which show that these compounds have considerable potential to cause acute toxicity in *C. riparius* larvae. While the sublethal effects, especially the biochemical effects, produced with the 10^7-fold lower concentrations of 24 h LC50, suggest that the biochemical parameters that were studied, such as the CAT activity, may have considerable potential as an early warning signal of chemical stress in *C. riparius*.

The increases in dry-body weights and emergence failure that occurred after EE exposure suggest that this chemical may perturb the physiological processes of *C. riparius*. The increase in dry-body weights is difficult to explain, but it could suggest growth stimulatory effect of BPA and EE. It may also be attributed to a decrease in larval water content, which is eventually related with the alteration of the larval osmoregulation process. However, the ash-free dry-body weights should be measured to determine the actual larval growth status.

A decrease in the emergence rate, an indicator of animal development, after exposure to the highest concentration of EE suggests that the alteration of this parameter may be considered the consequence of a serious progression of the toxic effect. Among the emerged adults, males and females were identified to verify whether there is any difference between the two in terms of their susceptibility to BPA or EE exposure. Although, any dose–response relationship was not found on the emergence of male and female, the increased male/female ratio after exposure to 1 μg/L BPA can be attributed to the greater vulnerability of female adults to BPA exposure compared to the male adults. More conclusive evidences are needed, though, to support this hypothesis since this phenomenon was not observed after exposure to higher concentrations of BPA.

Typically, BPA and EE are suspected to induce feminization in aquatic species. Sexual differentiation processes in animals, including arthropods are based on the ration of male and females sex hormones (Bogart, 1987). As vertebrate sex steroids are either absent in insects or have not been found to exert any specific effects (Svewers et al., 1991), a model is suggested in which ecdysone (*E*) acts both as direct precursor of the active molting hormone 20-hydroxyecdysone (20*E*) and as male sex steroid (De Loof and Huybrechts, 1998). Possessing estrogenic potential in vertebrates, BPA and EE may not alter invertebrate sex hormone related pathways, as in vertebrate, since we failed to find the evidence on feminization by the exposure of these compounds in *Chironomus*. On the contrary, slight increase in male/female ratio was observed by BPA exposure. The females seem to be more vulnerable than the males, in terms of success of the development; however, increased ratio of males/females in BPA-exposed *Chironomus* dose not mean male-dominant population.

Watts et al. (2001) showed that a chronic sediment test of BPA and EE on *C. riparius* revealed no consistent relationship between their effects on development and reproduction and chemical concentration. The results of the present study showed CAT and GST activation after BPA and EE exposure, and increased emergence failure after EE exposure. These data, however, are not sufficient to conclude that there is a correlation or casual relationship between them. Thus, direct experimental demonstrations of the wider relationships between the biochemical effects of BPA and EE exposure and their consequences at higher levels of biological organization, which is an ongoing project of these authors' laboratory, are needed to fully understand the effects of these compounds on *C. riparius* in particular and on the aquatic ecosystem in general. The characterization of the causal relationships between the biomarker responses of *C. riparius* and the effects of their BPA and EE exposure at higher biological levels will help define the sublethal hazards of chemicals in this animal.

In freshwater ecosystems, a complex mixture of pollutants frequently causes chemical pollution. This makes it considerably more difficult to predict the effects of

pollutants and emphasizes the need for studies on multiple biological endpoints to be conducted to identify pertinent biomarkers. The simultaneous measurement of various biological parameters will allow data to be obtained at different levels of biological organization and may help bring about a full understanding of the effects of a toxicant on organisms.

5. Conclusions

In this study, to investigate the short-term effects of BPA and EE on *C. riparius*, the biochemical and physiological parameters of *C. riparius* were investigated after their sublethal exposure to BPA and EE under controlled laboratory conditions. The results of the present study showed CAT and GST activation after BPA and EE exposure as well as increased emergence failure after EE exposure. These data, however, are not sufficient to establish any correlation or casual relationship between BPA/EE and the response of *C. riparius*. Thus, further research is required to come up with direct experimental demonstrations of the wider relationship between the biochemical effects of the two compounds on *C. riparius* and their consequences at higher levels of biological organization.

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