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Developmental Effects of Bisphenol a and Its Anologs Bisphenol S, Bisphenol F and Bisphenol Af on Sea Urchins *Paracentrotus lividus* (Lamarck 1816) and *Arbacia lixula* (Linnaeus 1758)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Restricting the use of BPA cause environmental concentrations of bisphenol S, bisphenol F, and bisphenol AF to increase. Because no data is available about the possible toxic effects of BPA analogues on sea urchin embryos, this study aims to investigate the developmental effects of bisphenol and its analogues on sea urchin embryos by embryotoxicity bioassay with two sea urchin species, *Paracentrotus lividus* and *Arbacia lixula*. The sea urchin bioassay may be performed on embryosand eggs, in well-standardized laboratory conditions. The embryos may be exposed to test agents throughout larval development, 72hrs. The exposure of eggs before fertilization may provide additional information on the ability of a chemical to induce teratogenic damage. Multispecies might be used in the tests because of the dissimilarity in susceptibility of different species to different contaminants. *P. lividus* and *A. lixula* embryos have been recognized as valuable tools in toxicological studies . In ecotoxicological studies, *P. lividus* have been more widely used than *A.*

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lixula A. lixula has not been widely used for toxicity testing, although in most of the studies *P. lividus* has been used. No comparative study has been carried out to reveal the differences in sensitivities between the two species to the pollutants. The EC50 (Effective Concentration 50) for 72 hours was determined at 1.396 mg-BPA/L for sea urchin *A. lixula* and 0.676 mg-BPA/L (EC50) was estimated BPA in P. lividus. The EC50 values for BPS, BPF, and BPAF for A. lixula were determined to be 2,673mg of BPS/L, 1,129 mg of BPF/L, and 0,314 mg of BPAF/L respectively. It can be concluded that these chemicals adversely affect the embryonic developmental stages of of P. lividus and A. lixula, which is of great ecological importance due to the hazard at the population level. In this way, the result of this study present the nominal effective concentrations of BPA and its analogues and the suitability of the species for use as a biomarker in ecotoxicology tests. In conclusion, the sea urchin model may usefully contribute to the identification and characterization of harmful animals. The advantages in using this test system should therefore prompt its extensive use in the biological monitoring of pollutants.

Keywords: Bisphenol A; Bisphenol analogs; Paracentrotus lividus; Arbacia lixula; toxicity.

1. INTRODUCTION

Plastic pollution poses a threat to marine ecosystems due to its widespread use in all areas. As a result, it has a variety of effects on aquatic organisms, many of which have yet to be studied [1]. The use of a wide variety of plastic products has increased considerably in recent years due to their social benefits such as ease of use, practicality, etc. Being durable and light, plastic has become the preferred base material for many applications, especially industrial ones. On the other hand, the multifaceted use of plastic has led to an increase in environmental pollution and a threat to natural life. Certain additives/chemical compounds are used in order to have the desired properties (durability, etc.) and to facilitate the production of plastics during the production phase. The most widely used of these compounds, bisphenol A (BPA), is used in the production of polycarbonate and epoxy resins [2]. BPA is commonly used as a stabilizer, an antioxidant in polycarbonate plastic [2,3]. BPA has a wide range of uses, such as food packaging, bottles, straws, thermal receipt paper, toys, CDs, and medical devices [4]. The burning and photodegradation of plastics cause BPA contamination in aquatic environments [5-7]. Because the decomposition of BPA occurs rapidly in UV light, heat, acidic, or basic environments, it causes pollution in the environment and human exposure to natural life [8]. The toxic effects of bisphenol, have received great attention because it acts as a xenoestrogen and causes endocrine disruption. Today, due to the ban on the use of BPA in many countries [9]. It has led to the rapid development of compounds with similar chemical and physical properties to BPA and the replacement of BPA and its analogues (such as Bisphenol S, Bisphenol F, Bisphenol AF). Bisphenol analogues with similar structures, whose effects and safety are uncertain, are released to the marine environment through plastic leaching [10]. Detectable levels of BPA range from 0.5-146 µg/L in freshwater rivers and industrial effluent [2]. There have been numerous studies that show BPA is toxic to fish and invertebrates (LC50 ranges from 1.1 to 10 mg/L) [2,11,12]. Because of a lack of data, particularly on its toxicity at low doses. Today, it is believed that the BPA alternatives are "safer". BPS and BPF are the second and third most abundant analogues in the environment, detected at even higher levels than BPA in surface waters [13]. A few studies have documented that BPS may be equally or more harmful than BPA [14]. So, new researchers advised that it was necessary to investigate the current alternatives used instead of BPA. Chen et al. [15] have identified the potentially toxic effects of BPA alternatives on non-target organisms. Furthermore, these analogues have also been determined to be endocrine-disrupting chemicals [13]. BPS, BPF, and BPAF are found in lower concentrations in water sediment [15-18] and bioaccumulate in the bodies of several animal species [19]. Restricting the use of BPA leads to a greater use of BPA alternatives and increases their production. Therefore, concentrations of BPA alternatives are expected to increase in all areas of the environment. (PNEC) was reported as 1500 ng/L by the European Union [20]. There is a lack of ecotoxicological data on the possible adverse effects of BPA analogues on aquatic invertebrates. It is well known that the effects of toxic chemicals on aquatic organisms are of great importance in the protection of the natural population's health. Sugni [21] suggested that echinoderms were good indicators and useful

test species in ecotoxicology. Echinoderms can supply more than 90% of the benthic zone, and their habitats assemble those points of environmental pollution so that they are key components of marine ecosystems [2,22,23]. Several laboratories around the world have successfully developed the use of sea urchin embryos and gametes in testing the developmental effects of chemicals [24,25]. *Paracentrotus lividus* is a species used as a bio-indicator in ecotoxicological studies that is distributed throughout the Mediterranean Sea [25]. *Arbacia lixula* has been found to share habitats with *P. lividus* in the Mediterranean [25]. They favor different substrates for feeding. P. lividus eats mostly algae and sea grass, whereas A. lixula eats sessile invertebrates [26]. As the preferred test organisms, sea urchins are available virtually throughout the year, the tests give speedy, easy, and sensitivity responses in a very short time, the cost is low, and they enable the sub-lethal effects to be determined [22-26]. The use of fertilization and offspring toxicity bioassays with a sea urchin to evaluate pollution of marine sediments and seawater for monitoring in coastal areas has been normalized with toxicity tests [26]. *A. lixula* has not been widely used for toxicity testing [23,25,26], although in most of the studies *P. lividus* has been used [26,27]. No comparative study has been carried out to reveal the differences in sensitivities between the two species to the pollutants. In the light of this information, in this study the embryotoxic effects of increasing concentrations of BPA (0.300-3.5 mg/L), BPS, BPF, and BPAF (0.2-12.0 mg/L) which are based on previously measured LC50 levels for other aquatic organisms), were tested on the embryos of the sea urchins *Paracentrotus lividus* (Lamarck, 1816) and *Arbacia lixula* (Linnaeus 1758). Although data from a single animal's acute toxicity testing may be considered as overly simplistic, it is frequently used to assess ecotoxicity [28]. As is well known, the toxicity of chemicals varies according to species.As it is well known the toxicity of chemicals ranged according to species. New studies have indicated that bisphenols affect ecosystem health and endanger environmental safety [29], but studies on the comparative toxicity of bisphenol analogues are limited. Although little data have been reported on the toxic effects of BPA, BPS, BPF, and BPAF on some aquatic species, there is a lack of information about the toxic effects on aquatic organisms and the embryotoxicity of sea urchins. There are no data on the developmental effects of BPS, BPF, and BPAF on P. lividus and A. lixula. Especially no data were available about the developmental effects of BPS, BPF, and BPAF on *P. lividus and A. lixula*. Therefore, in this study, we will investigate the influence of bisphenol A and different bisphenol analogues (BPS, BPF, and BPAF) on the development of sea urchins using the bioassay

2. MATERIALS AND METHODS

Analytical grade bisphenol-a [(CH3)2C(C6H4OH), Cas No: 80-05-7], Analytical grade bisphenol-s [(O2S(C6H4OH)2, Cas no: 80-09-1 (4,4'-Sulfonylbisphenol)], Analytical grade bisphenol-F [(CH2(C6H4OH)2, Cas no: 620-92-8] and Analytical grade bisphenol-AF [(CF3)2C(C6H4OH)2, Cas no: 1478-61-1] were purchased from Sigma-Aldrich, Germany. Test chemicals were dissolved in dimethylsulphoxide (DMSO) (Sigma, Cat. No: 67-68-5) as 100 µg-BPA, BPS, BPF, BPAF/L. Test concentrations for *P. lividus* were 0.3, 0.5, 0.8, 1.0, 1.5, 2.3 and 3.5 mg-BPA/L, 0.2, 0.5, 0.6, 0.8, 1.0, 1.5, 2.0, 3.0, 6.0 and 12.0 mg/L of Bisphenol S, Bisphenol F and Bisphenol in 10 ml of final test volume. For A. *lixula, t*est concentrations were 0.005, 0.1, 0.2, 0.6, 0 1.5 and 3.5 mg-BPA/L, 0.4, 0.6, 0.8, 1.0, 1.2, 1.5, 1.8, 2.0, 3.0 and 5.0 mg/L of Bisphenol S and Bisphenol F and 0.1, 0.2, 0.25, 0.3, 0.35, 0.4 and 0.5 mg/L of Bisphenol AF in 10 ml of final test volume. Controls accompanying the experiments were untreated negative controls (filtered natural seawater collected from the same area as the echinoids: FSW, 35 PSU) and 3x10-4M $CdCl^2$ as a positive control. Sperms and embryos were also exposed to DMSO as solvent control, being added to the medium in the same volume as the highest test concentration of chemicals, and no effect was detected on the development of sea urchin embryos. All treatments were tested as six replicates in six well-plates (Nunc, Denmark). Adult sea urchins, *Paracentrotus lividus*, and *Arbacia lixula* were collected from the Aegean Sea coast (Seferihisar District, Turkey). Sea urchin embryo bioassays were carried out as described previously by Pagano et al., [25]. For the embryotoxicity tests, the gametes of sea urchin were harvested and 1 ml fertilized egg suspension was added gently to contaminated FSW with increasing BPA, BPS, BPF, and BPAF concentrations and exposed to the medium from 10 minutes after fertilization up to the pluteus larval stage (throughout development) [25]. The room temperature was 18 ± 2°C for P. lividus, 19 ± 2°C for *A. lixula* during the experiments. Embryotoxicity was assessed on 72-hour-old

pluteus larvae according to morphological criteria defined by Pagano et al. [25]. A sample of 100 embryos was observed under a light microscope. Developmental defects were observed on living plutei, which slowed down their mobilization in 10–4 M chromium sulfate, 72 h after fertilization. The following outcomes were evaluated: retarded (R) plutei, pathologic malformed plutei (P1); pathologic embryos (P2) unable to differentiate up to the pluteus larval stages, and dead (D) embryos/larvae which are immobilized (Fig. 1).

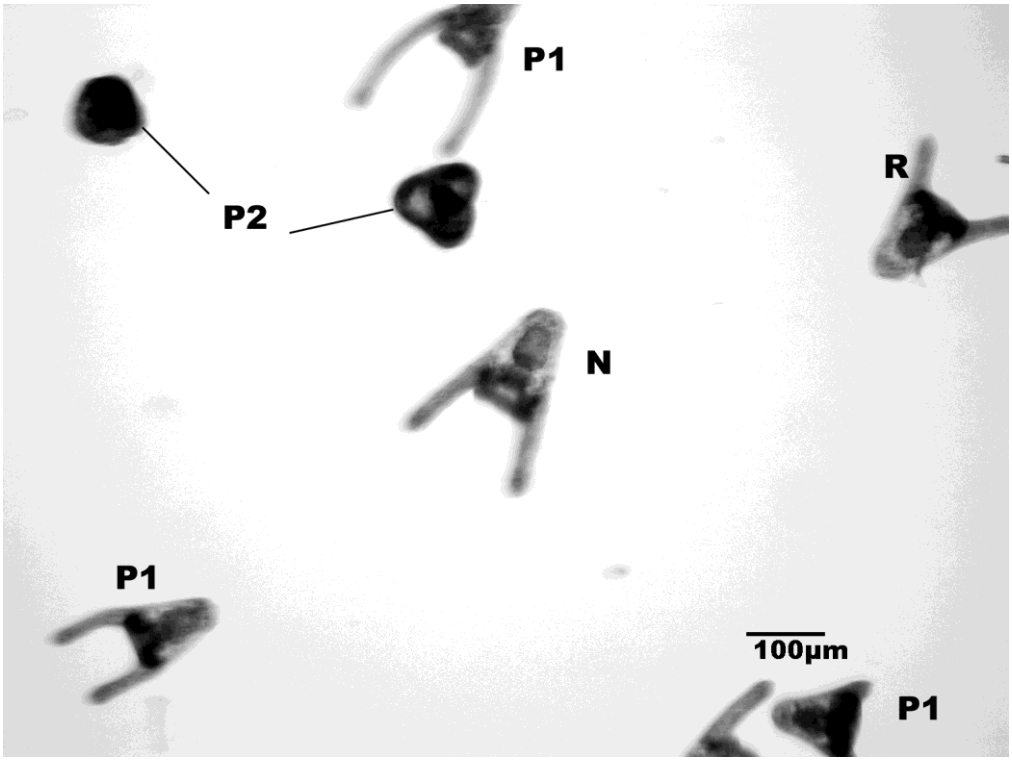

Fig. 1. Embryonic malformations (P1: skeletal malformations and gastrointestinal tract abnormalities; R: half size of a normal larvae; P2: Page 4/12 pre-pluteus stage blockage; D: early embryonic death)

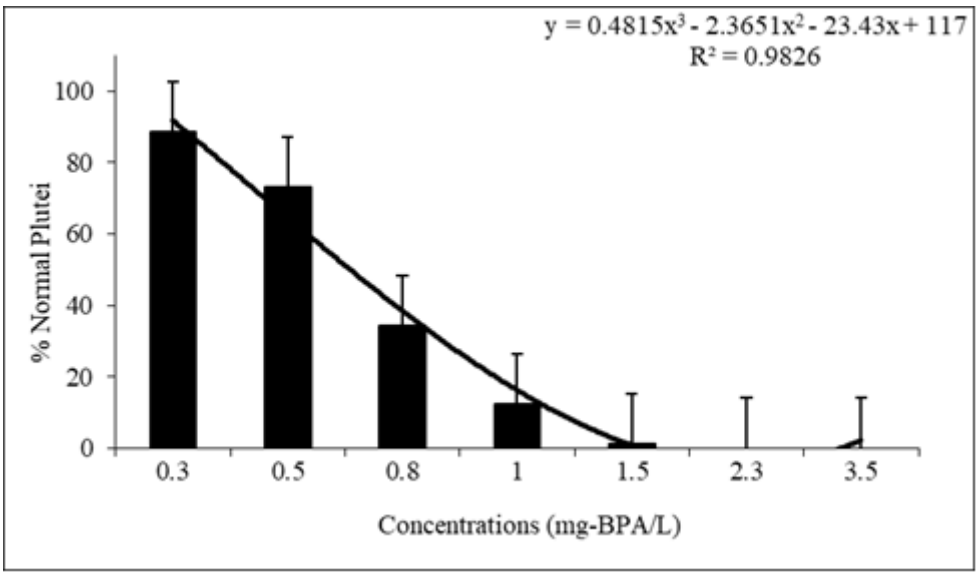

Fig. 2. Embryotoxic effects of Bisphenol-a on normal plutei frequencies of *P. lividus*

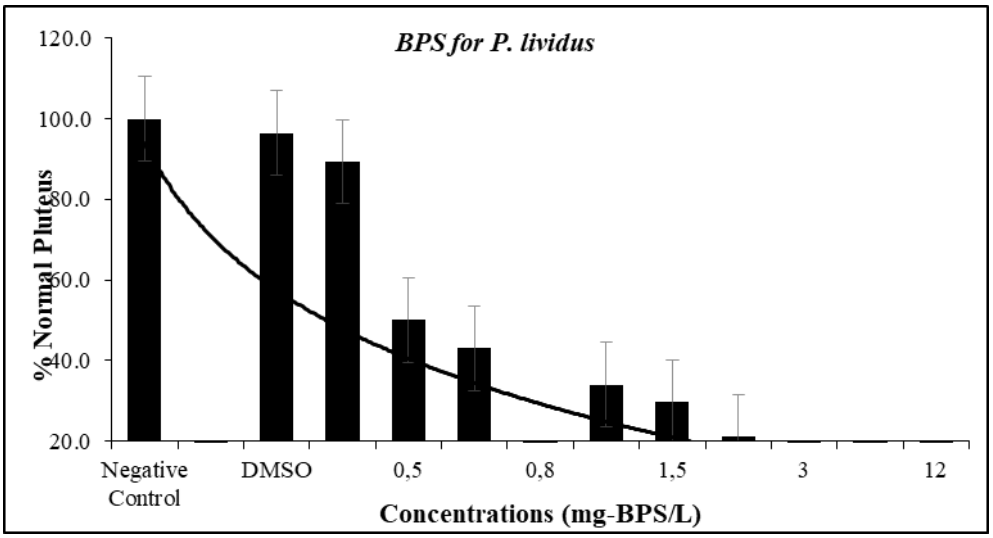

Fig. 3. Embryotoxic effects of Bisphenol- s on normal plutei frequencies of *P. lividus*

2.1 Statistical Analysis

EPA Probit Analysis Program used for calculating LC/EC Values Version 1.5. Student -t-tests were used to compare the differences in the frequency distribution of the evaluated parameters (N: normal plutei, R: retarded plutei, P1: skeletal malformations, P2: blocked gastrula or blastula, and D: dead) between the negative control (FSW) and the treatment groups by applying the logarithmic transformation to normalize distributions. For statistical analysis, the significance of differences in the distribution of the proportion of larval classes was tested by Student-t-tests and Dunnet test using the Statistica 6.0 statistical program [30].

3. RESULTS

When embryos were exposed to bisphenol-a and analogs, which are bisphenol S, bisphenol F, and bisphenol Af, throughout embryogenesis, significant effects of these compounds were observed at concentrations ranging from 0.2-12mg/L. The embryotoxicity tests show the classic dose-response curve, indicating a decreased percent of normal sea urchin (P. lividus and A. lixula) development with increasing bisphenols (Figs. 2-9).

Results of *Paracentrotus lividus* embryotoxicity: Probit analyses estimated the impact of BPA on exposed embryos to be EC500.676mg/LBPA concentration Table 3-4. On the other hand, the lower concentrations of BPA generally caused malformations in the skeleton (Table 1). Concentrations of 3.0 and 5.0 mg/L of BPA caused skeletal deformity rates of 7,5 % and 22, 89 %, respectively. Although no larval malformations (P1) were observed at 2.3 and 3.5 mg/L BPA, differentiation was arrested at the gastrula stage (P2) by approximately 63%. Larval malformations (P1) and arrest of differentiation at the gastrula stage (P2) reached approximately 97% for BPA at 1500 mg/L while the highest BPA concentrations (3.5 mg-BPA/L) caused mortality (reaching 100%). It has been seen that the lower concentrations of BPA caused skeletal deformities, while the higher concentrations of BPA adversely affected the embryos at earlier stages of development (Fig. 2).

By using probit analyses, the impact of BPS on exposed embryos was estimated to have as EC50 0.658 mg-BPS/L (Tables 3-4). The lower concentrations of BPS generally caused malformations in the skeleton (Table 1). Concentrations of 0.2 and 0.5 mg/L of BPS caused skeletal deformity rates of 10,5 % and 46 %, respectively. Although in 2.3 and 3.5 mg/L BPA larval malformations (P1) were not observed, the arrest of differentiation at the gastrula stage (P2) reached approximately 4%. Larval malformations (P1) and arrest of differentiation at the gastrula stage (P2) reached approximately 10% for BPS at 3.0g/L. It has been seen that the lower concentrations of BPS caused skeletal deformities while the higher concentrations of BPS adversely affected the embryos at earlier stages of development (Fig. 3).The embryotoxicity tests show the classic dose-response curve indicating a decreased percent of normal sea urchin (*P. lividus*)

development with increasing BPF (Fig. 4). The lower concentrations of the BPF generally caused malformations in the skeleton. When compared with the negative control group, concentrations of bisphenol-f between 0.2-12.0 mg-BPF/L caused skeleton deformities of 36% and 73 %, respectively ($p<0.001$) (Table 1). The impact of BPF on exposed embryos was estimated as EC50 0.430 mg/L BPF concentration by probit analyses Table 3-4. As a result of the embryotoxicity tests, the dose-response curve (Fig. 4) showed a decreased percentage of normal pluteus at BPF concentrations ranging from 0.2 to 12.0 mg-BPF/L when compared with the negative control group ($p<0.0001$). According to the dose-response curve, the lowest concentrations of BPAF influenced the development of P. lividus embryos. The scores of developmental defects of larvae generated by BPAF-exposed embryos showed that developmental hazards were significantly increased at all concentrations tested ($p<0.0001$) (Table 1, Fig. 5). By probit analyses, the effect of BPAF on exposed embryos was estimated to be EC 500.519 mg/LBPAF concentration Tables 3-4.

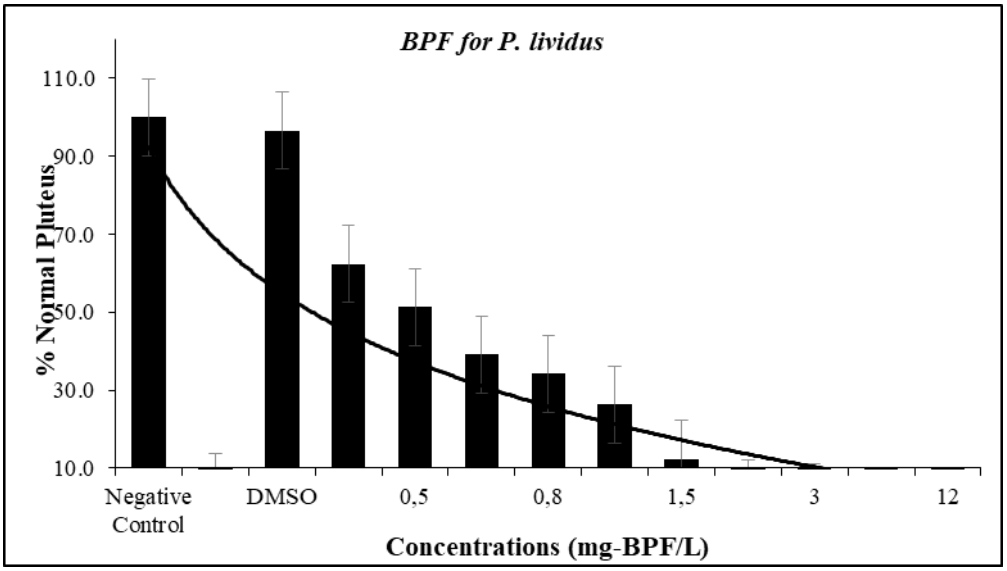

Fig. 4. Embryotoxic effects of Bisphenol-f on normal plutei frequencies of *P. lividus*

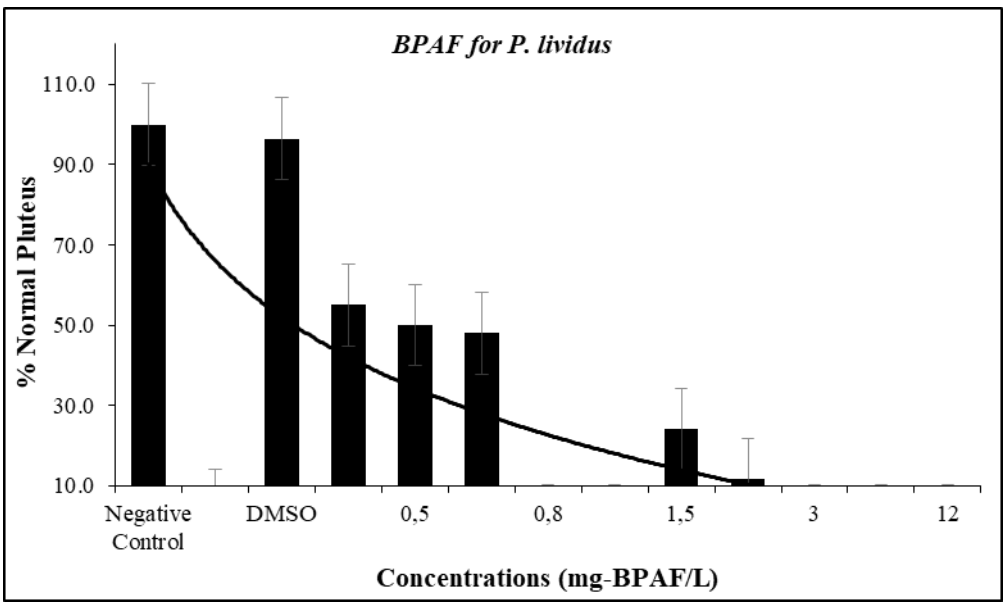

Fig. 5. Embryotoxic effects of Bisphenol-af on normal plutei frequencies of *P. lividus*

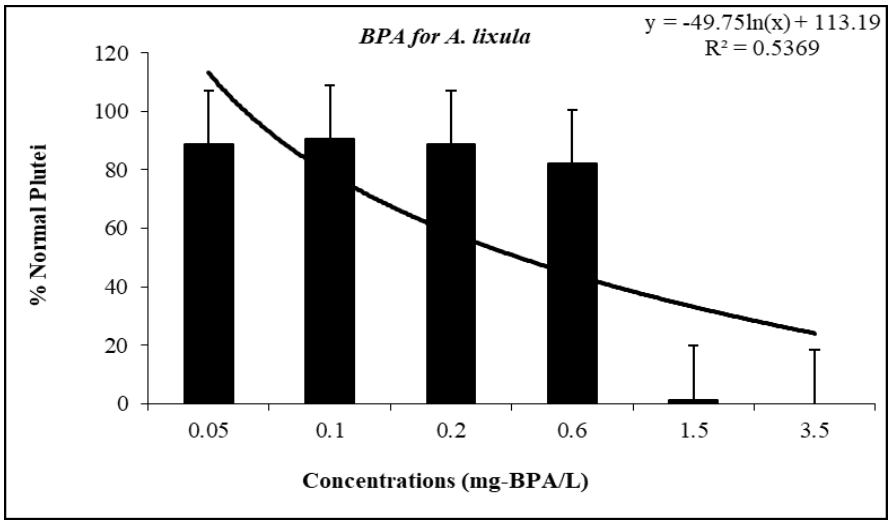

Fig. 6. Embryotoxic effects of Bisphenol-a on normal plutei frequencies of *A. lixula*

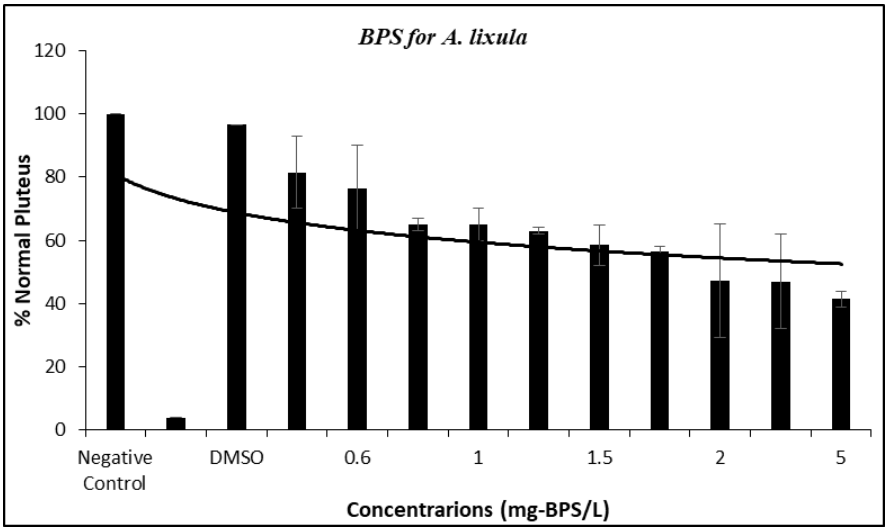

Fig. 7. Embryotoxic effects of Bisphenol- s on normal plutei frequencies of *A. lixula*

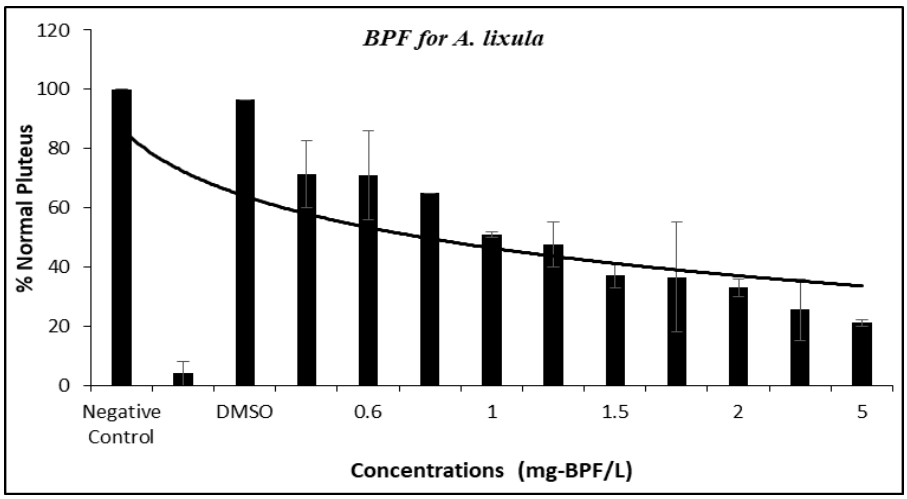

Fig. 8. Embryotoxic effects of Bisphenol-f on normal plutei frequencies of *A. lixula*

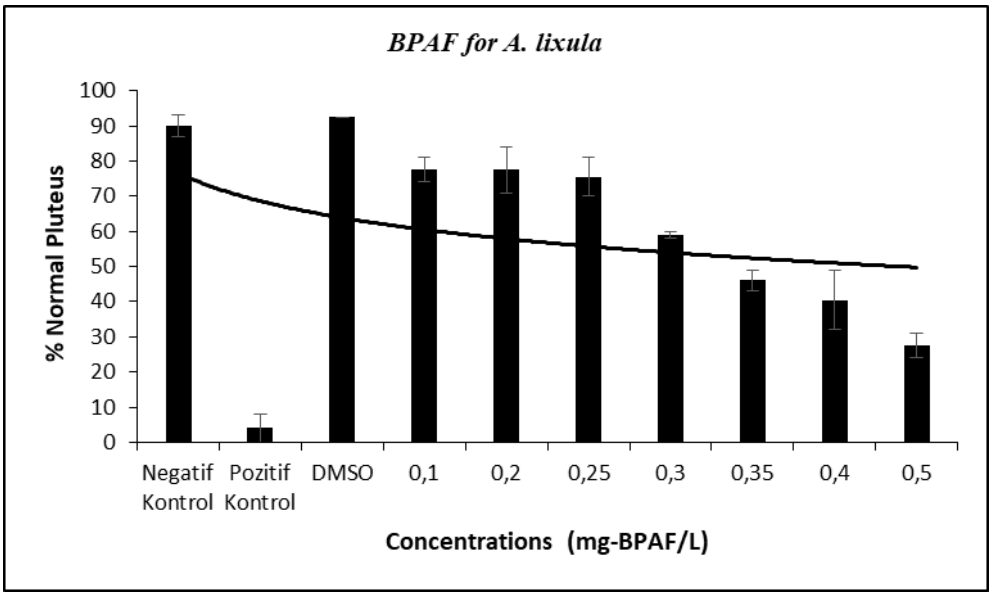

Fig. 9. Embryotoxic effects of Bisphenol-af on normal plutei frequencies of *A. lixula*

Results of *Arbacia lixula* embryotoxicity: When embryos were exposed to BPA, BPS, BPF, and BPAF just after fertilization and throughout embryogenesis, significant effects were observed at concentrations ranging from 0.05 to 5.0 mg/L. The embryotoxicity tests show the classic dose-response curve, indicating a decreased percentage of normal sea urchin (*A. lixula*) development with increasing BPA, BPS, BPF, and BPAF concentrations (Table 2, Figs 6-9). By using probit analyses, the impact of BPA on exposed embryos was estimated as to have an EC50 of 0.710 mg/L BPA concentration Table 3-4. On the other hand, the lower concentrations of BPS generally caused malformations in the skeleton. Concentrations of 0.4-5.0 of bisphenol S and bisphenol F caused skeletal deformity rates of 18.5 % - 29 % and 73.5 % - 58 % respectively (Table 2, Fig. 6-7). The estimated EC50 levels of BPS and BPF were calculated as EC 50: 2.673 mg-BPS/L, and EC50:1.129 mg-BPF/L (Table 3). The impact of BPAF on exposed embryos was estimated as EC50 0.314 mg-BPAF/L concentration by probit analyses Table 3. The lower concentrations of the BPAF (0.1 mg/L) generally caused malformations in the skeleton (Table 2). skeletal deformity rates of 21.5 % and 25 %, were caused by BPAF concentrations of 0.2 and 0.25 mg/L respectively. Retarded plutei (R), larval malformations , Larval malformations (P1), and arrest of differentiation at the gastrula stage (P2) reached approximately 55% for BPAF at 3.5 mg/L. It has been seen that the lower concentrations of BPAF cause developmental effects (Fig. 7). According to the results of the experiments, it was observed that all of the chemicals applied had a hazardous effect on embryo development of sea urchins *P. lividus* and *A. lixula*, depending on the concentration, and an increase in the deformity percentages calculated based on the dose-response curve in Figs. (2-8) and the calculated EC50 (Tables 3-4). Results of the study showed that toxic levels of the bisphenol analogues were BPF>BPAF>BPS>BPA, for *P. lividus* and BPAF>BPA>BPF>BPS for *A. lixula*, respectively. During the experiments, it was discovered that all chemicals used had a restricting effect on development as concentration increased. The dose-response curve of normal larva frequency fell in all concentrations of bisphenols, but developmental malformations (P1 and P2) increased (Table 1-2, Fig. 9). In this study, the toxicity of BPA to P. lividus embryos was lower than that of its analogs, with a high EC50 of 0.0325 mg BPAF/L after 72 hours of exposure, indicating that this BPA alternative is toxic to sea urchin embryos. However, the toxicity of BPF and BPS to embrios of A. lixula was lower than that of BPA in this study, with a high EC50 of 0.37 mg-BPAF/L after 72h of exposure showing that this BPA alternative is toxic to sea urchin embrios (Table 4).

4. DISCUSSION

BPA has been widely known as toxic for many aquatic species, and analogues such as BPF, BPAF, and BPS have been introduced as a new substitute for BPA. Our study demonstrated that

Table 1. Embryotoxic effect of BPA, BPS, BPF and BPAF on *P. lividus* (R: Retarded plutei, P1: Larval malformations, P2: Blocked gastrula or blastula, D: Dead)

Chem. (mg/L)		N	R	P1	P2	D
Blank		95.9±1.0	0.56±0.23	2.72±0.82	0.61±0.2	0.22±0.15
$CdCl_2$	$3x10^{-4}$	0±0	0±0	45.33±20.4*	53.5±21**	1.17±0.55*
DMSO	3.5	89.33±2.5	1.17±0.8	7.5±2.51	2±1.1	0±0
BPA(mg/L)	0.3	88.5±4	0.5±0.5	9.33±3.44 *	1.33±0.8 *	0.33±0.2
	0. 5	73±5.7	0±0	20±2.57**	2.5±1	4.5±3.6*
	0.8	34.2±5.1	0±0	58±3.30**	2.16±1.14	5.66±4.6*
	1.0	12.3±3.8	0±0	69.16±3.79**	17.3±6.5**	1.1±1.1
	1.5	1.3±1.3	0±0	34.16±4.01**	63±15.2**	1.5±0.7*
	2.3	0±0	0±0	0±0*	33.66±8.0**	66.3±8.0*
	3.5	0±0	0±0	0±0*	0±0	100±0**
BPS	0.2	89,3 ±1,74	0±0	6,67±0,56*	4 ±1,6	0±0
	0.5	50 ± 2	0±0	46±2.6*	4±1.7	0±0
	0.6	43 ± 8.1	0±0	53.7±7.9*	3.3±0.3	0±0
	0.8	42.7 ± 1.9	0±0	55.3±0.9*	2.7±1.8	0±0
	1.0	34 ± 6,66	0,3± 0,3	64,3 ± 7,9*	1,33 ± 1,80	0 ± 0
	1.5	29,66 ± 3,6	0 ± 0	64,7± 4,91*	3,66 ± 2,68	0 ± 0
	2.0	21,15 ± 1,3	0 ± 0	72 ± 3,21*	6,83 ± 1,4	0 ± 0
	3.0	3.7±1.9	0±0	93±2.1*	3.3±0.9	0±0
	6.0	0±0	0±0	4±0.6*	96±0.6	0±0
	12.0	0±0	0±0	98.3±1.7	1.7±1.7	0±0
BPAF	0.2	55±3.1	0±0	41.3±0.9*	4±4	0±0
	0.5	50±2	0±0	46±2.6*	4±1.7	0±0
	0.6	48 ± 12,70	0 ± 0	51,8± 12,6*	0,16 ± 0,16	0 ± 0
	0.8	43.7±5.5	0±0	49.3±6.1*	7±4.0	0±0
	1.0	43±8.1	0±0	53.7±7.9*	3.3±0.3	0±0
	1.5	24 ± 6,66	0,3± 0,3	72,3 ± 7,9*	3,33 ± 1,80	0 ± 0
	2.0	11,66 ± 3,69	0 ± 0	84,7± 4,91*	3,66 ± 2,68	0 ± 0
	3.0	0 ± 0	0 ± 0	94,5 ±2,85*	5,5 ± 2,85	0 ± 0
	6.0	0 ± 0	0 ± 0	92 ± 3,21*	8,83 ± 1,4	0 ± 0
	12.0	0 ± 0	0 ± 0	33,3± 5,98*	66,7 ± 5,59	0 ± 0
BPF	0.2	62,3 ± 3,78	0 ± 0	29,2± 3,79*	7,3 ± 6,48	1,1 ± 1,1
	0.5	51,33 ± 1,3	0 ± 0	14,16±14,0*	23 ± 15,17	1,5 ± 0,72
	0.6	39,12 ± 0,3	0 ± 0	26,3 ± 8,04*	31,66 ± 8,0	1,7 ± 0,72
	0.8	34,16 ± 5,09	0 ± 0	58 ± 3,30*	2,16 ± 1,14	5,66 ± 4,58
	1.0	26.3±7.1	0±0	62.3±3.8*	11±7.8	0±0
	1.5	12,3 ± 3,78	0 ± 0	69,2± 3,79*	17,3 ± 6,48	1,1 ± 1,1
	2.0	2,3± 3,78	0 ± 0	62,3 ± 2,8*	2421,3 ± 6,4	1,1 ± 1,1
	3.0	1,33 ± 1,33	0 ± 0	58 ± 3,30*	36,16± 1,14	1,5 ± 0,72
	6.0	0 ± 0	0 ± 0	34,16±14,0*	63 ± 15,17	5,66 ± 4,5
	12.0	0 ± 0*	0 ± 0	0 ± 0	33,66 ± 8,0	66,33 ± 8,04

(p<0.005)

Table 2. Embryotoxic effect of BPA, BPS, BPF and BPAF on *A.lixula.*(R: Retarded plutei, P1: Larval malformations, P2: Blocked gastrula or blastula, D: Dead)

Chem. (mg/L)		N	R	P1	P2	D
Blank		100±0	0±0	0±0	0±0	0±0
$CdCl_2$	$3x10^{-4}$	0±0*	0±0	0±0	100±0	0±0
DMSO	3.5	96,5±0	0±0	3,5±0	0±0	0±0
BPA(mg/L)	0.3	89.3±1.1*	0±0	6.2 ± 0.9	0±0	0±0
	0.5	91.3±1.3*	0±0	3.3±0.8	6 ± 1.5	0±0
	0.8	88.7 ± 1.9*	0±0	3.2 ± 0.8	8.2 ± 2	0±0
	1.0	82.3 ± 3.3*	0±0	9 ± 2.2	8.7 ± 1.5	0±0
	1.5	1.3 ± 1.3*	0±0	47.7 ± 12.8	51± 12.8	0±0
	2.3	0.1± 0.1	0±0	97.4 ± 0.6*	2.6 ± 0.6	0±0
	3.5	0 ± 0*	0±0	0±0	0±0	100±0
Bisfenol-S	0,4	81,5±11,5*	0±0	18,5±11,5*	0±0	0±0
	0,6	76,5±13,5*	0±0	22,5±14,5*	1±1*	0±0
	0,8	65±2*	0±0	33,5±1,5*	1,5±0,5*	0±0
	1	65±5*	0±0	35±5*	0±0	0±0
	1,2	63±1*	0±0	37±1*	0±0	0±0
	1,5	58,5±6,5*	0±0	33,5±1,5*	8±8*	0±0
	1,8	56,6±1,5*	0±0	43,5±1,5*	0±0	0±0
	2	47,33±17,9*	0±0	52,66±17,9*	0±0	0±0
	3	47±15*	0±0	53±15*	0±0	0±0
	5	41,5±2,5*	0±0	57,5±1,5*	1±1*	0±0
Bisfenol-AF	0,1	77,5±3,5*	5±5	17,5±8,5*	0±0	0±0
	0,2	77,5±6,5*	1±1	21,5±7,5*	0±0	0±0
	0,25	75,5±5,5*	10,5±6,5*	13,5±1,5*	0,5±0,5*	0±0
	0,3	59±1*	21,5±2,5*	19,5±3,5*	0±0	0±0
	0,35	46±3*	15,5±15,5*	37±14*	3,5±3,5*	0±0
	0,4	40,5±8,5*	17,5±8,5*	42±17*	0±0	0±0
	0,5	27,5±3,5*	12±12*	58,5±17,5*	1±1*	0±0
Bisfenol-F	0,4	71,33±11,3*	0±0	28,66±11,2F	0±0	0±0
	0,6	71±15*	0±0	28±15*	1±0*	0±0
	0,8	65±0*	0±0	35±0*	0±0	0±0
	1	51±1*	0±0	45±3*	4±2*	0±0
	1,2	47,5±7,5*	0±0	48,5±3,5*	4±4*	0±0
	1,5	37±4*	0±0	60,5±5,5*	2,5±1,5*	0±0
	1,8	36,5±18,5*	0±0	63±18*	0,5±0,5	0±0
	2	33±3*	0±0	57±5*	10±2*	0±0
	3	25,5±10,5*	0±0	74±11*	0,5±0,5	0±0
	5	21±1*	0±0	73,5±4,5*	5,5±5,5*	0±0

(p<0.005)

Table 3. Estimated LC/EC values and confidence limits of BPA and its analogs on *Paracentrotus lividus* for Embryotoxicity

Paracentrotus lividus			
Estimated LC/EC Values and Confidence Limits of			
Exposure Point		**95% Confidence Limits**	
	Conc.(mg/L)	**Lower-**	**Upper**
BPA (mg/L)			
LC/EC 1.0	0.286	0.218	0.344
LC/EC 5.0	0.368	0.298	0.426
LC/EC 10.0	0.421	0.352	0.477
LC/EC 15.0	0.461	0.393	0.516
LC/EC 50.0	0.676	0.621	0.725
LC/EC 85.0	0.993	0.925	1.082
LC/EC 90.0	1.087	1.006	1.202
LC/EC 95.0	1.243	1.134	1.411
LC/EC 99.0	1.600	1.410	1.918
LC/EC 1.00	0.056	0.020	0.106
LC/EC 5.00	0.116	0.051	0.189
LC/EC 10.00	0.170	0.086	0.258
LC/EC 15.00	0.220	0.121	0.319
LC/EC 50.00	0.658	0.494	0.822
LC/EC 85.00	1.970	1.550	2.755
LC/EC 90.00	2.553	1.946	3.828
LC/EC 95.00	3.749	2.694	6.308
LC/EC 99.00	7.708	4.868	16.390
BPF (mg/L)			
LC/EC 1.00	0.041	0.012	0.081
LC/EC 5.00	0.081	0.031	0.140
LC/EC 10.00	0.117	0.052	0.188
LC/EC 15.00	0.151	0.073	0.229
LC/EC 50.00	0.430	0.301	0.551
LC/EC 85.00	1.231	0.981	1.663
LC/EC 90.00	1.578	1.228	2.280
LC/EC 95.00	2.281	1.684	3.707
LC/EC 99.00	4.550	2.960	9.480
BPAF (mg/L)			
LC/EC 1.00	0.026	0.002	0.081
LC/EC5.00	0.063	0.008	0.152
LC/EC10.00	0.100	0.018	0.213
LC/EC15.00	0.138	0.031	0.270
LC/EC50.00	0.519	0.263	0.775
LC/EC85.00	1.960	1.318	3.807
LC/EC90.00	2.684	1.729	6.192
LC/EC95.00	4.276	2.510	13.107
LC/EC 99.00	10.241	4.849	55.740

Table 4. Estimated LC/EC values and confidence limits of BPA and its analogs on *Arbacia lixula* for Embryotoxicity

Arbacia lixula			
Estimated LC/EC Values and Confidence Limits of			
ExposurePoint	**95% Confidence Limits**		
	Conc.(mg/L)	**Lower- Upper**	
BPA (mg/L)			
LC/EC 1.0	0.273	0.205	0.333
LC/EC 5.0	0.362	0.289	0.422
LC/EC 10.0	0.420	0.347	0.480
LC/EC 15.0	0.464	0.392	0.523
LC/EC 50.0	0.710	0.650	0.765
LC/EC 85.0	0.1087	1.008	1.192
LC/EC 90.0	0.1202	1.106	1.340
LC/EC 95.0	0.1396	1.263	1.599
LC/EC 99.0	0.1846	1.609	2.244
BPS (mg/L)			
LC/EC 1.00	0.013	0.002	0.035
LC/EC 5.00	0.061	0.020	0.120
LC/EC 10.00	0.141	0.061	0.234
LC/EC 15.00	0.248	0.129	0.368
LC/EC 50.00	2.673	2.153	3.652
LC/EC 85.00	28.842	15.043	86.693
LC/EC 90.00	50.634	23.564	185.441
LC/EC 95.00	116.566	45.752	572.959
LC/EC 99.00	556.896	158.420	4765.133
BPF (mg/L)			
LC/EC 1.00	0.029	0.012	0.053
LC/EC 5.00	0.084	0.044	0.132
LC/EC 10.00	0.149	0.089	0.214
LC/EC 15.00	0.220	0.142	0.298
LC/EC 50.0	1.129	0.983	1.282
LC/EC 85.00	5.793	4.451	8.409
LC/EC 90.00	8.530	6.198	13.469
LC/EC 95.00	15.135	10.094	27.144
LC/EC 99.00	44.361	25.092	101.478
BPAF (mg/L)			
LC/EC 1.00	0.008	0.000	0.039
LC/EC 5.00	0.024	0.000	0.072
LC/EC10.	0.043	0.000	0.100
LC/EC 15.00	0.062	0.001	0.126
LC/EC 50.00	0.314	0.205	0.677
LC/EC 85.00	1.573	0.707	422.414
LC/EC 90.00	2.304	0.886	2073.188
LC/EC 95.00	4.056	1.232	21990.619
LC/EC 99.00	11.715	2.269	1856746.375

Please adhere to internationally agreed standards such as those defined by the International Organization of Standardization (ISO). Metric SI units should be used throughout; L: liter; °C: degree; gr: gram; min: minute; h: hour; m: mili; n: nano; p: pico; f: femto; a: atto

BPA and its analogs can negatively affect the long-term development of *P. lividus* and *A. lixula*. Previous studies reported reduced or delayed fertilization after BPA exposure in humans [31], mice [32] and sea urchins [2,22]. Throughout the experiments, we observed consistent decreases in the normal development of embryos exposed to concentrations of both BPA and its analogs. We observed increased abnormalities in the embryos exposed to the chemicals, and that there was a dose-dependent increase in abnormalities with increasing concentrations of

the chemicals. There have been multiple studies that demonstrate these same findings [33]. Because no data is available about the possible toxic effects of BPA analogues on sea urchin embryos, an important part of the aquatic community. To investigate whether the bisphenol analogues cause developmental anomalies, the sea urchin embryotoxicity bioassay was assessed with two sea urchin species, *Paracentrotus lividus* and *Arbacia lixula*.As a result of the experiments, it was observed that all of the chemicals applied had a hazardous effect on the embryonic development of sea urchin *P. lividus* and *A. lixula*, depending on the concentration, and an increase in the deformity percentages calculated based on the dose-response curve in Figure (1-7) and the calculated EC50 (Table 3). Results of the study showed that toxic levels of the bisphenol analogs as BPAF>BPF>BPS>BPA, for *P. lividus* and BPAF>BPA>BPF>BPS for *A. lixula*, respectively. During the experiments, it was discovered that all chemicals used had a restricting effect on development as concentration increased. It has been reported by several researchers that BPA is acutely toxic to aquatic animals (LC50 ranged from 2.5 to 6900 mg/L) [2,22]. Also, the LC50 for BPA has been determined for a variety of aquatic organisms, including freshwater and saltwater algae, invertebrates (daphnids, sea urchin, and mysid shrimp), and fish with reported values ranging from 1000 to 20,000 µg/L [34]. According to Uibel et al. [1] BPA has a significant impact on the normal development of sea urchins Strongylocentrotus purpuratus when gametes and embryos are exposed to environmentally relevant concentrations.. Reports vary on the sensitivity of aquatic invertebrate organisms to BPA. For example, sea urchin (H*emicentrotus pulcherrimus*) embryos developed normally but grew significantly smaller adult sizes compared to controls in BPA concentrations as high as 570 µg/L [35]. Ozlem and Hatice [2] observed similar developmental abnormalities if the sea urchin *Paracentrotus lividus* embryos were exposed to BPA concentrations of 300µg/L. Our results are consistent with this study since the high concentrations (300-3500 µg/L) of BPA had a similar effect on *P.lividus*. According to research data by Karaaslan [36], Propranolol, which is widely used as a beta-blocker for cardiovascular diseases, and methylparaben, one of the most abundant ingredients in pharmaceuticals, are entering the aquatic systems together, so they are both detected in the environment up to mg/L levels, adversely effected sea urchin *P. lividus* embryos.

5. CONCLUSION

In our investigation, BPA and its congeners were tested for their potential adverse effect on the development of sea urchin embryos of *P. lividus and A. lixula.* The findings revealed that the parameter of developmental effects in sea urchin embryos raised concern about the toxicity of BPA and congeners.. Our results and the results of other related studies showed that contamination with BPA and its analogs caused a developmental effect. The results of the investigations on the developmental effects of BPs on sea urchins are not available. These types of studies are important for predicting the toxic effects of chemicals on living organisms. Consequently, according to the report of Chen et al., [11], BPA and its analogs concentrations in several aquatic environments are lower than even the estimated EC10 values. Our results and previous studies show that BPA concentrations in the environment may not be hazardous at present unless to keep the environmental concentration of BPs is kept under control. Because it is vital to the sustainable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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