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Research Article

Ecotoxicological risk of bisphenol Z and bisphenol A diglycidyl ether: A comparative study on *Phaeodactylum tricornutum* and *Mytilus galloprovincialis* in aquatic environments

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Abstract

Studies on the ecotoxicological effects of bisphenol analogues, such as bisphenol Z (BPZ) and Bisphenol A Diglycidyl Ether (BADGE) are still sparse. These compounds can still be widely found in the environment due to it recalcitrance, lipophilicity, and bioaccumulation. This study aimed to investigate the ecotoxicological and cytotoxic effects of BPZ and BADGE on *P. tricornutum* and *M. galloprovincialis* to compare the produced effects with the sum of the effects caused by each substance. We also intend to establish a positive correlation between the results obtained from the *P. tricornutum* and *Mytilus* assays for each tested compound, also sharing information regarding the cell replicates exposed to the toxicants. The experimental data will allow the estimation of harm caused by these toxicants in the *M. galloprovincialis* earlier life stage, contributing to an environmentally safe future application of these compounds. Comprehending these intricate effects is crucial for a comprehensive ecological risk assessment and necessitates further investigation into the mechanisms behind the detrimental effects of BPZ and BADGE on marine ecosystems.

Introduction

The bisphenols are a family of typically synthetic industrial precursors used to produce many different polymers that are found in a variety of products. In the context of the ongoing effort to replace bisphenol-A (BPA), aimed at minimizing the negative effects on biological systems, two new, frequently used bisphenols have arisen: bisphenol Z (BPZ) and bisphenol A diglycidyl ether (BADGE) (Han *et al*., 2021). BPZ and BADGE stand out as two prominent compounds among the analogues of BPA extensively utilized in industrial products. They were developed due to the numerous adverse effects, even at remarkably low concentrations, of BPA on both living organisms and human health (Schmidt, 2013). Recently, BPZ and BADGE have taken on additional meaning in biomedicine, bioactive epoxy composites, and research with tetrafunctional monomers, including BADGE. BPZ has become a hot topic due to the development of new biocompatible materials for applications in biomedicine, electronics, or in the oil and gas industry. While BPZ is utilized in personal care products, food packaging, paper products, and in the synthesis of anesthetic chemicals such as fensclidine (PCP) (Schmidt *et al.,*2013; Han *et al*., 2021), BADGE serves as a monomer in the production of epoxy resins and is employed in the coating material for food and beverage containers, anti-corrosive coatings, and exterior composites (Chamorro-Garcia *et al*., 2012; Szczepanska *et al*., 2019; Miyazaki *et al*., 2020; Zhang *et al*., 2021). Despite the advantages of BADGE because of the their physicochemical properties, recent studies have shown the negative effects of these xenobiotics on the growth, development, and physiological status of aquatic organisms. Although the increasing production and usage of these analogues are not fully known, research indicates their presence in surface waters and sediments in marine and freshwater ecosystems, ranging from ng to µg/ L (Czarny-Krzymińska *et al*., 2023).

BPA analogues have reach to the aqutic environment through during the production, treatment, processing of bisphenols in wastewater treatment facilities, and household solid waste (Liu *et al*., 2021; Huang *et al*., 2021; Godiya and Park, 2022). In comparison to BPA, the insufficiently determined potential effects of BPZ and BADGE in aquatic ecosystems cast doubt upon them (Parlak *et al*., 2011). Considering the log Kow values determined for BPZ 5,4 and BADGE 3,48, it suggests their biological accumulation and binding to organic matter. The physicochemical properties of BPZ and BADGE contribute to predicting their environmental fate but individually do not provide sufficient data. possbilty

The potential risk to the marine environment posed by the released bisphenols raises the need to test the ecotoxicity of these substances in order to set the maximum allowable environmental levels for bisphenols. From the reports of mutagenic, estrogenic, and aquatic toxicity of bisphenols, such as; BPA, BPS, BPZ have been removed from consumer products. Today, they are used mainly in industrial applications, care products, and some industrial coatings. Other compounds, such as BPZ and BADGE are

also likely to have similar advantages and disadvantages. The compounds released from these resins are believed to be endocrine disruptors and cause a variety of negative effects on biological systems. These compounds are not included in relevant laws to protect humans and aquatic ecosystems from their direct adverse effects. Research on the toxicity of BPZ and BADGE on aquatic organisms will enable us to formulate guidelines for monitoring these foreign compounds.

Physicochemical processes and chemical analyses serve as indicators in determining the environmental fate of chemicals; however, the selection of ecotoxicological tests and test organisms is essential to predict their effects and mechanisms in aquatic environments. Ecotoxicological studies provide a good opportunity to identify potential effects on organisms at each trophic level following the entry of pollutants/chemicals into the environment. Moreover, ecotoxicological studies aid in determining action plans necessary for preserving, sustaining, and ensuring the safety of entire aquatic ecosystems. In assessing the quality of aquatic environments, fish are widely preferred as bioindicators for determining the effects of environmental pollutants (Ates *et al*., 2008). The potential utility of biomarkers for monitoring both environmental quality and the health of organisms living in polluted ecosystems has received increasing attention in the last few years (Selamoglu *et al*., 2015). Assessment of biochemical and hematological parameters provides valuable information about the physiological response to environmental changes. Sublethal doses administered to animals provide a clear understanding of the critical levels of certain pollutant chemicals. Fish in particular are commonly used to estimate the influence of environmental pollution due to the sensitivity of their biochemical and hematological parameters under such conditions (Selamoglu *et al*., 2015; Caglar *et al*., 2019). The study sought to test the chronic effects of harmful substances that have repercussions on reproduction and population dynamics; such problems should not be insubstantial (Ates *et al.,* 2008). A decrease in the primary producer's ability will lead to changes in the entire ecosystem (Lalli and Parsons, 1993). In this research, two contaminants were studied, synthesized, and used in significant amounts. The results demonstrate enhanced toxicity in analogue BPZ and BADGE. This demonstrates that algal growth inhibition tests can provide useful data for each Micro marine organism (MMO) target. They can be aggregated into a higher energy evaluation system, for example, a mussel culture or fish breeding system with temperature controls, feeding, and light–dark circadian cycles of different organisms to assess these emerging contaminants (Gibson *et al*., 1990; Stefels and van Baekel, 1993). Therefore, they are regarded as initial organisms in ecotoxicological studies, used to determine the initial response to the effects of new chemicals (Parlak *et al*., 2011).

Bivalves, as primary consumers at another trophic level, are widely used as test organisms in ecotoxicological studies. Due to their widespread geographical distribution, sedentary lifestyle, and limited

mobility, bivalve species reflect the conditions of their habitats and have been termed 'sentinel species'. Mussels are top consumers and often adopt a sedentary lifestyle linked to the availability of suitable habitats. For these reasons, the monitoring of *M. galloprovincialis* populations and their health can be a valuable tool for inferring the status of the surrounding ecosystem (Farrington *et al*., 2016). The assessment of the response of this species to environmental conditions is a useful tool in bioassessment studies, as it allows for a certain amount of overlap between bioassessment and biomonitoring (Chiesa *et al*., 2018). In recent years, the scientific community has been focusing on the use of mussels as part of environmental monitoring programs and as the basis for the development of new marine protected areas. Mytilus spp. have been used for the monitoring of several chemicals and pollutants and have played a key role in several estuarine and coastal monitoring programs (Świacka *et al*., 2019). Many studies concerning both natural and innovative monitoring methodologies for *M. galloprovincialis* indicate the integrative potential of this species within the ecological status as provided by the European Water Framework Directive. The dominant position of *M. galloprovincialis* in most benthic ecosystems might provide information on the ability of environments and communities to continue to provide humanity with the ecosystem goods and services. The use of these mussels as bioindicators would likely support the objectives of sustainable marine management (Staniszewska *et al.*, 2017). The use of mussels in environmental pollution research extends to pharmaceuticals (Świacka *et al*., 2019), microplastics (Li *et al*., 2019), and other emerging pollutants such as endocrine-disrupting compounds (Chiu *et al*., 2018). Specifically, bivalves are considered model organisms as sentinel species for determining the effects of endocrine-disrupting compounds and monitoring their environmental status (Omar *et al*., 2019).

The Algal Growth Inhibition Test, also referred to as the algal growth test, uses photosynthetic, planktonic, and benthic algal species to examine the influence of physical and chemical agents on the algae. The rapid growth and high surface area-to-volume ratio of many algal species make them particularly susceptible to both acute and long-term exposure to xenobiotics. Many industries produce large numbers of unknown substances that consume considerable resources to fully evaluate (Parlak *et al*., 2011). The Algal Growth Inhibition Test is a significant tool in evaluating potential adverse effects of various chemicals on aquatic ecosystems. Its primary objective lies in assessing the sensitivity of phytoplanktonic organisms, primary producers in aquatic ecosystems, to specific chemicals or environmental stressors. This test determines the inhibitory effects on the growth of phytoplanktonic organisms by exposing them to varying substance concentrations, expressed through endpoints such as growth rate or biomass. Understanding the effects of chemicals on phytoplanktonic organisms provides general information about the overall health and functioning of aquatic ecosystems. Studying the physiological impacts of chemicals on the

species *Mytilus galloprovincialis* holds significant importance in environmental research as it sheds light on how various chemical compounds impact the physiology of this species, which plays a fundamental role in marine ecosystems. Investigating these effects aids in assessing potential risks posed by pollutants, including their impacts on physiological parameters such as condition index, gonadosomatic index, and reproductive success. This research is vital for comprehending the broader implications of chemical contamination on marine biodiversity and ecosystem dynamics. Additionally, studying these effects provides valuable insights into the health and resilience of marine organisms, helping gauge the overall ecological health of aquatic environments. In this context, the ecotoxicological effects of BPZ and BADGE were evaluated on *P. tricornutum* and *M. galloprovincialis*, two species holding significant positions in the aquatic ecosystem at various trophic levels.

Materials and methods

Bisphenol Z (BPZ; $C_{18}H_{20}O_2$) was purchased from Sigma-Aldrich, purity %98 (Cas. No: 843-55-0, Molecular weight: 268, 36 g/mol). Bisphenol A diglycidyl ether with a %98 purity (BADGE; $C_{21}H_{24}O_4$) was purchased from Sigma-Aldrich (Cas No: 1675-54-3) and dissolved in pure water. The stock solutions were stored in dark vials. Intermediate stocks were prepared and utilized by dilution for the tests.

Algal growth inhibitions

Phaeodactylum tricornutum was chosen as the marine species for the assays due to its widespread presence as a phytoplankton in marine environments. The algae were cultivated in the laboratory using the f/2 medium at a temperature of 20°C, exposed to continuous white light of approximately 100 µmol photon m^{-2} s^{-1} within a 12:12 h light: dark cycle. Stock cultures were maintained in filtered (pore size: 0.2 µm) and autoclaved natural seawater enriched with f/2 medium (OECD, 1984) on a rotary shaker operating at 125 rpm.

To assess the toxicity of BPZ and BADGE on the growth rate of the marine phytoplankton diatom *P. tricornutum*, six replicates were exposed to increasing concentrations of compounds for 72±2 hours. Test concentrations for algal growth inhibition were selected as 0.5- 0.8- 1.0- 1.5, and 2.0 mg/L. Cellular density was determined using a Bürker counting chamber (Karl Hecht KG, Sondheim, Germany) under a light microscope (Bx51 Olympus, Japan). The algae were cultured until reaching the exponential growth phase before being transferred to a new culture medium for subsequent testing. Growth inhibition concentrations and their respective 95% confidence limit values were estimated through a linear regression model after natural logarithm data transformation of the measured cell density. Endpoints of the study were assessed based on cell count data and the calculated growth rate (0 to 72h) as per standard protocols (OECD 1984), derived from the mean cell counts of each test series. The average specific growth rate (µ) for exponentially growing cultures was calculated using the formula:

$$\mu 0\text{-}j = \ln x_j - \ln x_0 / t_j - t_0 \ (day^{-1})$$

Where, μ0-j: growth rate, X_0: initial number of cells / ml at time t_0, Xj: measured number of cells/ml at tj, Tj: time of the first measurement after the test began.

The percentage inhibition of cell growth (%Ir) at each test substance concentration was calculated as the difference between the control growth curve (μc) and the growth curve at each test substance concentration (μt), using the formula:

%Ir = μc - μr / μc x 100

Statistical analysis

The IC_{50} values, indicating the concentration causing 50% growth inhibition compared to the control treatment, based on growth rate, were calculated from the inhibition-concentration curve using the "area under the curve" method prescribed by the OECD (1984). IC_{50}-values were determined by nonlinear regression analysis. All results are presented as mean± SD, with significance considered at p<0,05. The data analysis utilized Statistica-6.0 software (Dunnett and Tamhane, 1991), and comparisons of growth rates with controls were conducted using the Dunnett's test. The LC_{50} value was calculated using the EPA Probit analysis program version 1.5. After determining the Condition and Gonadosomatic index values with the formula, ANOVA was applied in the Statistica 12.0 program.

Mussels acute, gonadosomatic-condition index and fertilization tests.

Mussels were collected from a clean area that is not exposed to any domestic or industrial wastewater, in the İzmir-Çeşme (Aegean coast of Turkey 38°27′ 00.01 N″–26°37′ 27.60 E″). Before the experiment, all mussels were taken from a stock tank (260×80×70). They were acclimatized (one week) in the laboratory in tanks of artificial seawater (After purchasing Natural Sourching sea salt cas number: 7647-14-5, This product does not meet the definition of a hazardous substance or preparation as defined by the European Union Council Directives 67/548/EEC, 1999/45/EC, 1272/2008/EC and subsequent Directives) in the laboratory environment) (5.1±0.1 mg/L dissolved oxygen, 8.1±0.1 pH and 34.5±0.2 psu.) aerated continuously at 17.5±1°C. In the experiment, 20lt volume glass aquariums were used.

After the acclimatization period, the initial step was the conduct of an acute toxicity test. Artificial seawater was introduced into glass aquariums in the test setup, followed by the installation of aeration. Subsequently, 20 mussels were placed in each glass aquarium. Throughout the entire trial, the temperature was maintained at 16°C, while monitoring oxygen and pH levels to ensure optimal conditions. For determining the acute effect of BPZ and BADGE on mussels, five additional concentrations (2, 4, 8, 16, 32 mg/L) were chosen in addition to the control. Over a duration of 96 hours, the number of dead and surviving mussels in each aquarium was observed and recorded every 24 hours. T The LC_{50} value was calculated using the EPA Probit analysis program version 1.5.

To ascertain the physiological effects of BPZ and BADGE on mussels, growth and gonadosomatic index tests were conducted. After the acute test, the trial duration was extended to 14 days with the surviving

mussels (n=10). At the end of the trial, which utilized the same experimental setup and test concentrations (2, 4, 8, 16, 32 mg/L), the mussels were dissected.

The impact of BPZ and BADGE on the Condition Index (CI) was determined following the criteria by Matozzo *et al*. (2012). Soft tissues were collected by separating the shells from four individuals. Both shells and soft tissues were dried in an oven at 60°C for 48 hours. Subsequently, calculations were performed using the formula provided below based on the measured weights of the soft tissues and shells:

CI (%) = (dry tissue weight (g)) / (dry shell weight (g)) ×100

The Gonadosomatic Index (GSI) was determined according to Peters and Granek (2016). Dissection was performed on four individuals, and gonads were separated from all tissues and dried in an oven at 60°C (48 h). Later, calculations were carried out using the formula provided below based on the measured weights of gonads and soft tissues:

GSI (%) = (Dry weight of gonads (g)) / (total dry weight of soft tissues (g)) ×100

The effect of BPZ and BADGE on fertilization success was investigated by placing mussels brought to the laboratory environment individually in glass jars. The water temperature was naturally raised to induce the release of sperm and eggs, ranging from 16°C to 22°C. Subsequently, the release of eggs and sperm was observed. Three healthy six female and six male individuals, determined by microscope observation, were selected. After identifying male and female individuals distinguished by a change in water colour, eggs and sperm were respectively filtered through 100 µm and 55 µm filters. Following the selection of three healthy male and female individuals based on criteria set by His *et al*. (1999) test concentrations (0.01, 0.1, 1, 100, 1000 ng/L) were chosen according to the calculated LC_{50} values. Stock solutions prepared for each chemical were diluted and used accordingly. In a 6-well plate, 9 ml of artificial seawater and increasing chemical concentrations were added, followed by an egg: sperm ratio of 1:10. After 30 minutes, fertilization success was fixed with 4% formalin for microscopic observation. Fertilized eggs were distinguished by the formation of the fertilization membrane. Fertilization success was evaluated based on the Fertilization Ratio (FR = % fertilized eggs). Corrected indices (CI) were calculated by comparing the observed fertilization ratio in the toxicant group (FO obs) with that in the negative control group (FO control) (Arslan and Parlak, 2008):

CI= (FO observed- FO control) / FO control ×100

The corrected index facilitates an easy observation of the relative increase (CI> 0) or decrease (CI <0) in fertilization success for each concentration.

Results

This study aimed to assess the impact of Bisphenol Z (BPZ) and Bisphenol A diglycidyl ether (BADGE) on the marine model organisms *P. tricornutum* and *M. galloprovincialis* from an ecotoxicological perspective. To examine the potential

effects of BPZ and BADGE using the model organism *P. tricornutum*, an algal growth inhibition test was conducted. The control group exhibited a 1.5-fold increase in phytoplankton cell count at days 0, 24, 48, and 72, as per OECD (1984) criteria, indicating the healthy progression of the test. No adverse effects were observed in our phytotoxicity trials within the control group. Evaluation of the restrictive/toxic effects of BPZ and BADGE on phytoplankton was based on microscopic cell counts of phytoplankton at 0, 24, 48, and 72 hours, compared with the control group (without toxic substances). The graph depicts the impact of increasing concentrations of BPZ (0.5-0.8-1-1.5-2 mg/L) on the growth rate of *P. tricornutum* over a 72-hour exposure period. While the initial concentration of 0.5 mg/L displayed an effect on *P. tricornutum*, a low inhibitory effect was observed at three concentrations (0.8, 1, 1.5 mg/L) of BPZ. An increase in the inhibitory effect was noted at a concentration of 1 mg BPZ/L. The calculated IC_{50} value for BPZ after 72 hours was determined to be 5.16 mg/L (Fig.1).

A significant difference was detected between the changes in growth rate compared to the control and also between the IC50 values determined depending on the concentration (p<0.05). Effects of BPZ and BADGE on *P. tricornutum* were tested by Algal growth inhibition test and IC50 (EC50) levels were calculated as 5,16 mg/L for BPZ and 11,71 mg/L for BADGE.

The figure displays the impact on the growth rate of *P. tricornutum* when exposed to increasing concentrations of BADGE (0.5, 0.8, 1, 1.5, 2 mg/L) over a 72-hour period. It was observed that at an initial concentration of 0.5 mg/L, the effect on *P. tricornutum* was minimal, whereas as the concentrations of BADGE increased, the inhibitory effect became more pronounced. The IC_{50} value calculated for BADGE after 72 hours was determined to be 11.71 mg/L (Fig. 2).

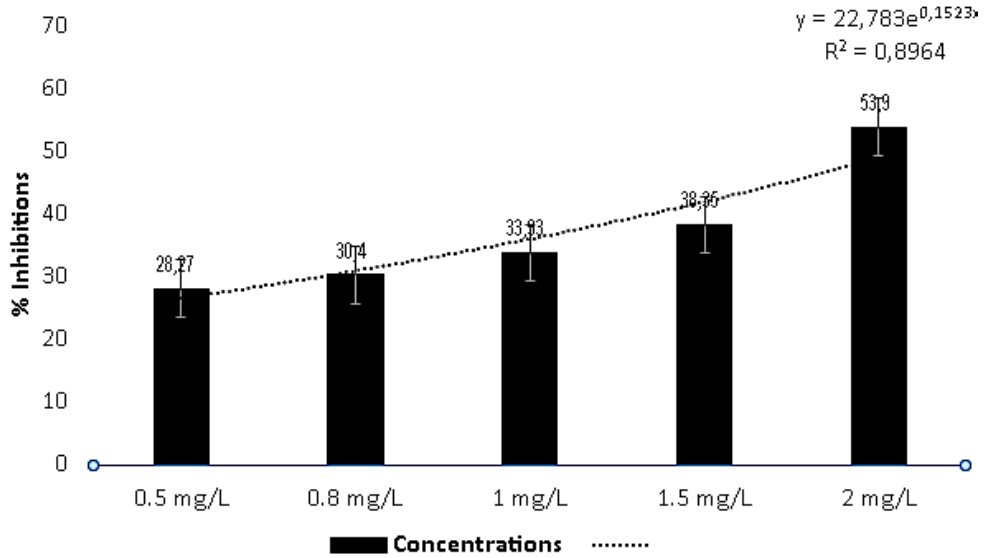

Figure 1: Inhibitions values (%) according to applied BPZ concentrations.

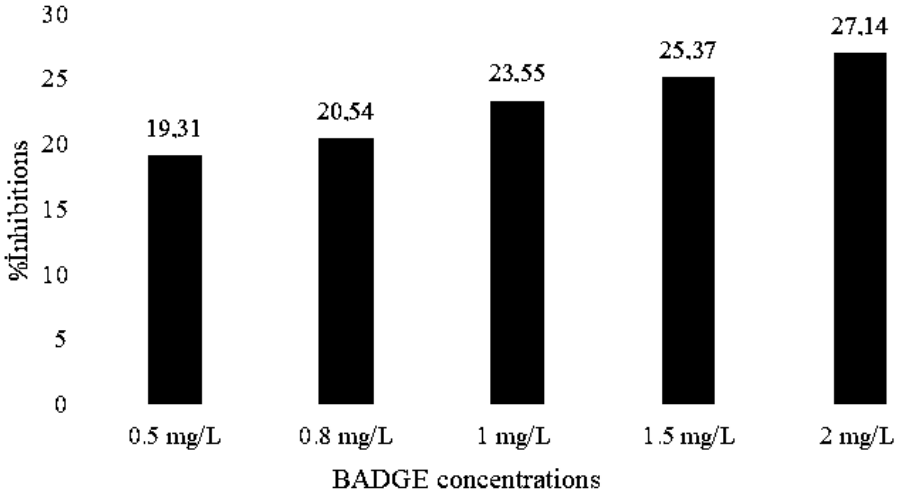

Figure 2: Inhibitions values (%) according to applied BADGE concentrations.

A significant difference was detected between the changes in growth rate compared to the control and also between the IC50 values determined depending on the concentration (p<0.05).

When comparing the IC50 values calculated from the concentrations applied to the model marine organism *P. tricornutum*, it was found that BPZ induced a higher level of inhibition compared to BADGE. The potential impacts of BPZ and BADGE on the marine model organism *Mytilus galloprovincialis* were evaluated. An acute toxicity test was conducted on adult individuals exposed to escalating concentrations of BPZ and BADGE (2, 4, 8, 16, 32 mg/L), resulting in the determination of the LC_{50} values as 11.24 mg/L and 15.40 mg/L, respectively.

CI % values of mussels exposed to increasing BPZ concentration were determined. A decrease in CI% values was observed compared to the control. It was determined that CI decreased to 8% at 2 mg/L BPZ concentration and to 6% at 32 mg/L BPZ concentration. While a decreasing trend was observed in all concentrations compared to the control (16%) (p<0.05), no significant difference was observed between the concentrations when compared to each other (Fig. 3). Exposure to increasing BPZ concentrations was determined to decrease GSI to 57% at a 2 mg/L BPZ concentration, causing a 42% decrease at the highest concentration (32 mg/L) (Fig. 4).

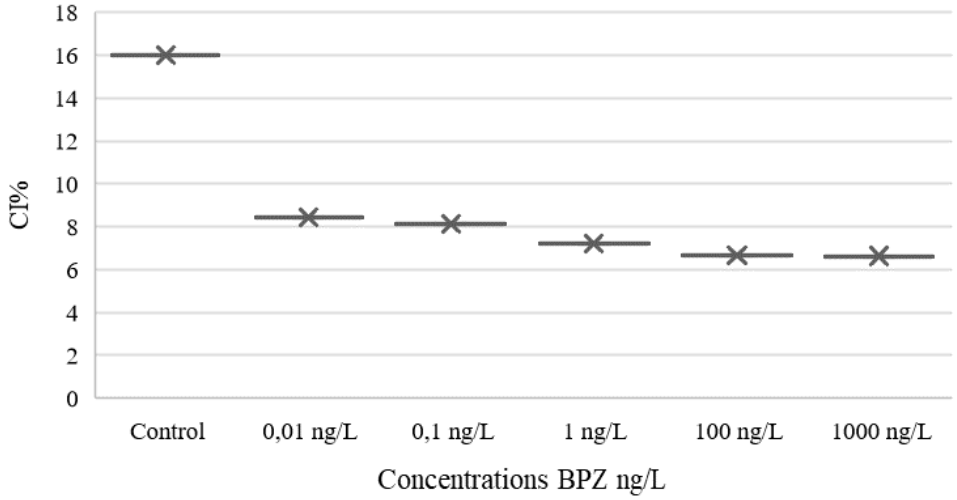

Figure 3: CI values (%) according to applied BPZ concentrations.

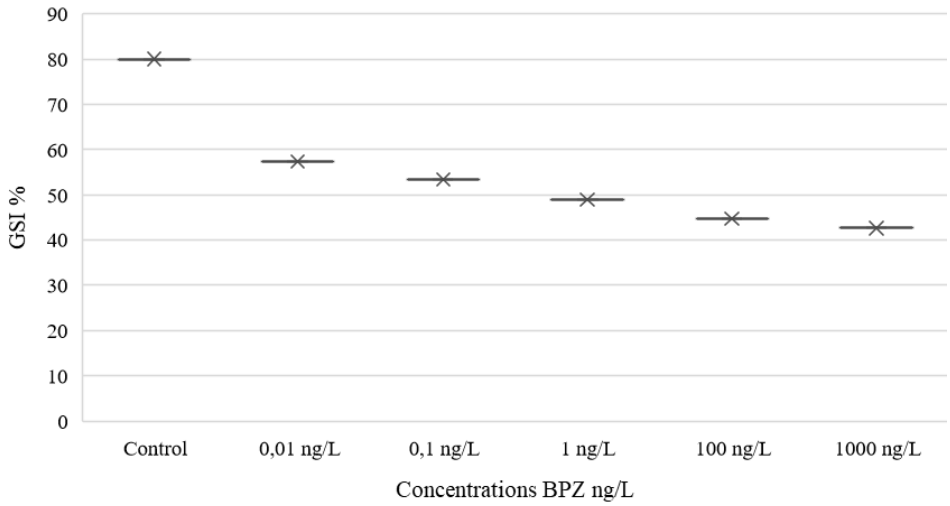

Figure 4: GSI values (%) according to applied BPZ concentrations.

A significant difference in %GSI was detected for increasing BPZ concentrations compared to the control (p<0.05). CI% values of mussels exposed to increasing BADGE concentration were determined.

A decrease in CI% values was observed compared to the control. It was determined that CI decreased to 9% at 2 mg/L BADGE concentration and to 4% at 32 mg/L BADGE concentration. While a decreasing trend was observed in all concentrations compared to the control (16%) (p<0.05), no significant difference was observed between the concentrations when compared to each other (Fig. 5). Exposure to increasing BADGE concentrations was determined to decrease GSI to 72% at a 2 mg/L BADGE concentration, causing a 40% decrease at the highest concentration (32 mg/L) (Fig. 6). A significant difference was detected in the GSI % determined with increasing BADGE concentrations compared to the control (p<0.05).

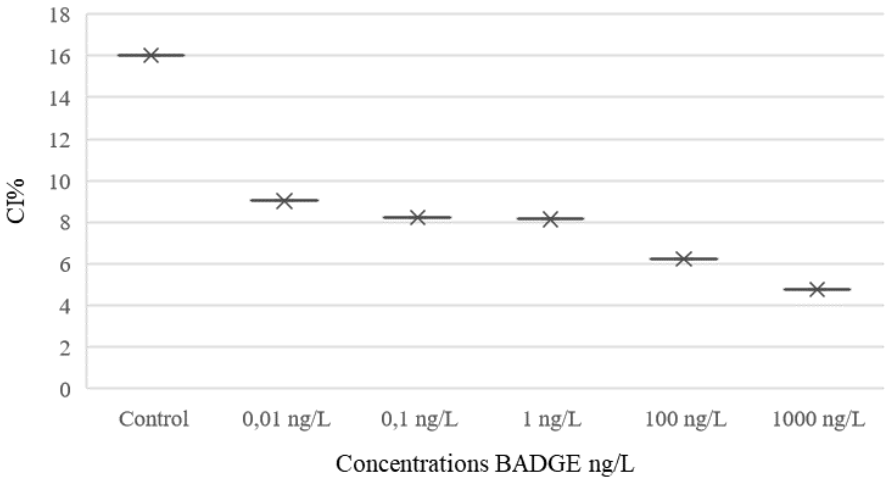

Figure 5: CI values (%) according to applied BADGE concentrations.

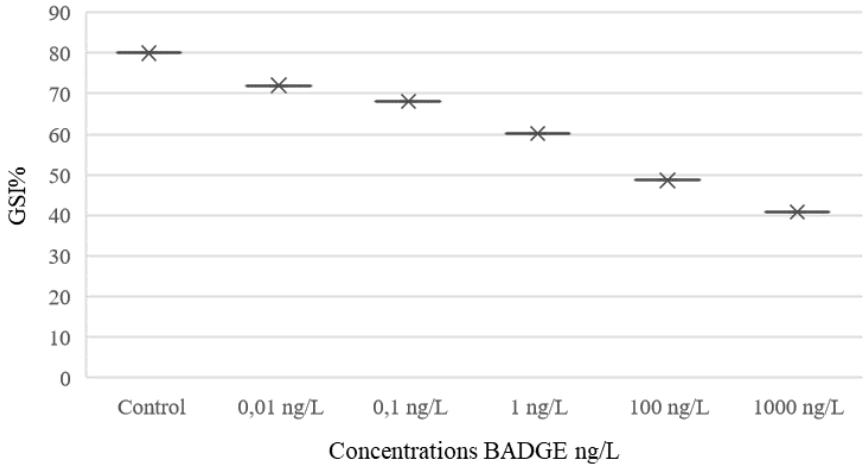

Figure 6: GSI values (%) according to applied BADGE concentrations.

The evaluation revealed that both BPZ and BADGE had an impact on the condition index and gonadosomatic index of *M. galloprovincialis* species. A comparative analysis between the two analogues demonstrated that BADGE exhibited higher efficacy concerning both the condition index and gonadosomatic index values. To evaluate the impact of BPZ and BADGE on mussel fertilization success, concentrations ranging from 0.01-0.1-1-100-1000 ng/L were administered. Comparative analysis against the control indicated minimal effects of both chemicals on fertilization at the lowest concentrations (0.01-0.1 ng/L), while their negative influence on fertilization success became more pronounced at higher concentrations (Fig. 7).

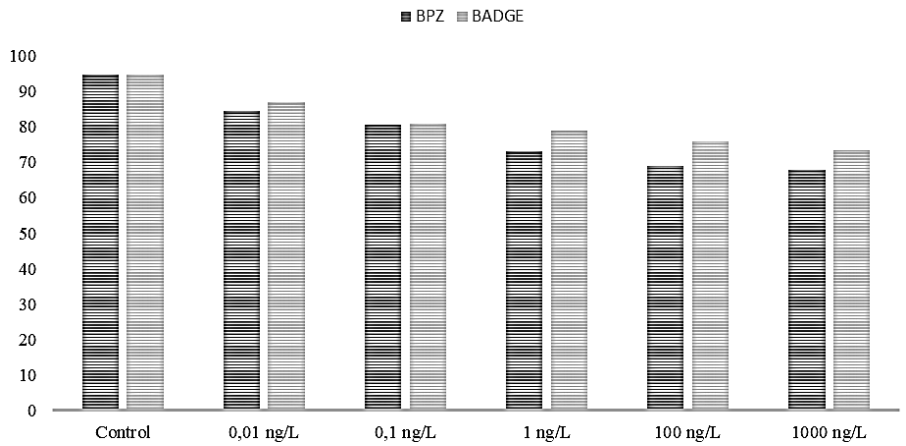

Figure 7: *In vitro* fertilization success rate based on applied BPZ and BADGE concentrations.

However, while BPZ notably decreased fertilization success at concentrations of 100 and 1000 ng/L, a similar rapid decline was not observed with BADGE. Although BADGE generally affected fertilization success with increasing concentrations compared to the control, the reduction was less pronounced compared to BPZ. Table 1 presents the DE values for each analogue, highlighting that BPZ exhibited a more substantial negative effect than BADGE, leading to a reduction in fertilization success when comparing the two analogues.

Table 1: Corrected indices % calculated based on applied BPZ and BADGE concentrations.

Control	Concentrations (ng/L)	% FO	CI	Unfertilized egg
		94.67± 0.88		**5.33 ± 0.88**
BPZ	0.01	84.5 ± 1.5	-10.73	15 ± 1.5
	0.1	80.5 ± 1.5	-14.96	19 ± 1.5
	1.0	73 ± 2	-22.88	27 ± 2
	100	69 ±1	-27.11	31 ± 1
	1000	68±1	-26.33	32 ± 1
BADGE	0.01	87 ± 1	-8.09	13 ± 1
	0.1	81± 2	-12.27	19 ± 2
	1.0	79 ± 1	-12.91	21 ± 1
	100	76 ±1	-13.59	24 ± 1
	1000	73.5±0.5	-14.00	26.5 ± 0.5

Discussion

The effects of BPZ and BADGE on marine model organisms, *P. tricornutum* and *M. galloprovincialis*, were investigated and evaluated ecotoxicologically. Information regarding the harmful effects of Bisphenols, compared to higher trophic level organisms, on planktonic organisms that affect the proper functioning of aquatic ecosystems is limited. The lipophilic nature of many BPs (log Kow) has been reported to allow them to penetrate the cell membrane of microorganisms, leading to various physiological damages such as growth inhibition, lipid peroxidation, or photosynthesis (Czarny *et al*., 2021). In our investigation, comparative analysis of the 72-hour EC_{50} values for *P. tricornutum*

revealed that both BPZ and BADGE induce less toxicity compared to BPA. However, when assessing the toxicity between the analogues, BPZ exhibited a higher level of toxic effect than BADGE. The 96h-EC_{50} value for BPA in *P. tricornutum* was reported as 0.6 mg L^{-1} (Seoane *et al*., 2021). Studies comparing the effects of BPA and its analogues on various phytoplanktonic organisms have been conducted. For instance, in one study examining *Chlorella vulgaris* species, it was observed that BPA (EC_{50}=39.80–44.90 mg/ L) and its analogues(BPAF, BPAP, BPG, and BPS) (EC_{50}=3.16- 28.99 mg/ L) demonstrated higher levels of toxicity (Ding *et al.,* 2020). Conversely, BPM, BPP, and BPZ (48.76–19930.34 mg L^{-1}) were reported to have fewer toxic effects (Czarny *et al*., 2021). Our findings regarding BPZ align with this data. Notably, there is currently no available algae study conducted with BADGE, preventing any comparison with other phytoplanktonic organisms. The effects noted in studies involving BPA and its analogues likely stem from variations in susceptibility among phytoplanktonic organisms and differences in their cell wall structures and compositions. Consequently, the specific effects of BPA and its analogues are unique to each species. Factors such as exposure duration, concentration, and biological characteristics of the species also significantly contribute to these effects. However, it's crucial to recognize that aquatic ecosystems possess intricate structures, and chemicals can induce synergistic or antagonistic effects. These substances undergo diverse processes in aquatic environments based on their physicochemical properties. These alterations not only modify their mechanisms of action but also influence the responses of living organisms. Thus, investigating the toxic impacts of chemical mixtures is imperative to accurately estimate ecological risks. The LC_{50} and EC_{50} values, specific to individual species, serve as indicators for identifying concentrations that reveal sublethal effects. These values may vary between adults and larvae of the same species. For instance, Tato *et al*. (2018) reported an EC_{50} value of 2085 µg/L for BPA in *M. galloprovincialis* larvae. Compared to BPA, our studyrevealed lower LC50 values for BPZ and BADGE in adults; this indicates less acute toxicity effects. It reveals that larvae show greater sensitivity compared to adults. However, our primary goal was to uncover physiological changes in mussel surviving acute toxicity tests. Therefore, the exposure duration for surviving mussels were extended to 14 days. Subsequently, increased BPZ and BADGE concentrations led to a decline in physiological parameters, notably the condition index (CI) and gonadosomatic index (GSI). These indices are widely employed in coastal monitoring studies to assess conditions in contaminated regions. Studies have previously reported elevated CI% values in both contaminated areas and mussel breeding zones, indicating the positive influence of heavy metals in polluted areas (Andral *et al*., 2004). Similarly, other research documented a reduction in both CI% and GSI% due to carbamazepine exposure in *M. galloprovincialis* (Oliveira *et al*., 2017). Our study revealed that exposure to BPZ and BADGE led to diminished CI% and

GSI% in *M. galloprovincialis*. Exposure to BPZ and BADGE resulted in decreased CI%, negatively affecting growth, and a decline in GSI. Mussels likely reduced their feeding activity by filtering to avoid BPZ and BADGE uptake. It's presumed that the energy not utilized for metabolism and reproduction was redirected toward defense and repair mechanisms, potentially reducing their bioaccumulation capacity and leading to various effects. Determining the sublethal impacts of various pollutants/chemicals is pivotal in ecotoxicological studies as it aids in predicting risks at the population, community, and ecosystem levels. In our investigation, aside from assessing the lethal effects of BPZ and BADGE on *M. galloprovincialis*, we aimed to evaluate their potential impact on fertilization success as a non-lethal effect. While BPZ and BADGE didn't adversely affect fertilization success at low concentrations, a negative influence was observed at higher concentrations, leading to reduced fertilization success. Upon comparing the analogues, it was evident that BPZ had a more pronounced negative effect on mussel fertilization success compared to BADGE. Several studies have delved into the effects of BPA on mussel embryos. For instance, in a study where *M. galloprovincialis* was exposed to BPA (0.05, 0.5, 5 µM), shell development was assessed at various stages after larval exposure. The research concluded that BPA exposure significantly impacted shell development, causing calcification issues. At the highest concentration, a significant number of larvae failed to reach the normal D-veliger stage, resulting in elevated malformation rates (Miglioli *et al*., 2021). Although it's established that BPA exerts a toxic effect on embryos, there is a lack of similar studies focusing on its analogues. However, it's reasonable to assume that, akin to BPA, the detrimental impact of BPZ and BADGE on fertilization success may potentially affect embryo development.

Conclusion

Aquatic products are essential nutrients in human nutrition and are also available to consumers in the global aquaculture industry. Therefore, we need to protect our aquatic environment from pollution from various environmental and ecological effects. Aquatic ecosystems and living organisms suffer from environmental impact due to emissions of volatile organic substances and pollution of water by petroleum chemicals and many other hazardous agents. In summary, the ecotoxicological assessment uncovered potential risks associated with Bisphenol Z and Bisphenol A diglycidyl ether on two marine model organisms, *Phaeodactylum tricornutum* and *Mytilus galloprovincialis*. BPZ and BADGE were found to inhibit the growth of *P. tricornutum*. Additionally, both analogues induced physiological effects in *M. galloprovincialis*, negatively impacting fertilization success. Considering the positions of these species in the trophic levels, the presence of BPZ and BADGE may pose risks to aquatic ecosystems. Such risks may disrupt population balances of species and potentially affect other trophic levels, thereby posing a threat to human health. Consequently, based on our study's findings, it's crucial to conduct more

comprehensive investigations on both analogues. These studies should consider varying concentration levels and the potential higher toxicity of analogues in mixtures. These measures are necessary before conclusively determining that the current concentrations observed in aquatic ecosystems won't result in toxic effects.

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Conflicts of interest

The authors have no conflict of interest.

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