Bisphenol S leads to cytotoxicity-induced antioxidant responses and oxidative stress in isolated rainbow trout (Oncorhyncus mykiss) hepatocytes

Burak Kaptaner1 · Can Yılmaz2 · Handan Aykut1 · Emine Doğan1 · Ceylan Fidan2 · Müşerref Bostancı1 · Fatoş Yıldız1

Received: 28 May 2021 / Accepted: 16 September 2021 / Published online: 13 October 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract

Background Bisphenol S (BPS) is a chemical compound that is utilized in the plastic industry as an alternative to bisphenol A (BPA). The toxic effects of BPS in fish is less known and limited. Therefore, in the present study, the influence of BPS on rainbow trout (Oncorhyncus mykiss) hepatocytes in vitro was investigated.

Methods and results For this purpose the fish hepatocytes were isolated, and then the cultured cells were treated with increasing concentrations of BPS (0, 15.63, 31.25, 62.50, 125, 250, and 500 µM) for 24 h. The cytotoxic impact of BPS was determined in the culture media using lactate dehydrogenase assay and then, the antioxidant defence indicators were assayed. The results showed that concentration-dependent increases were observed in the percentage of cytotoxicity. The superoxide dismutase activity was reduced, while the catalase and glutathione peroxidase activity increased with all of the BPS concentrations. The glutathione S-transferase (GST) activity significantly increased after a BPS concentration of 31.25 µM or higher, while GST Theta 1-1 activity was decreased by the same concentrations of BPS. The reduced glutathione content significantly decreased with a BPS concentration of 31.25 µM or higher, and the malondialdehyde content increased after BPS concentrations of 125, 250, and 500 µM.

Conclusions The findings determined herein suggested that BPS causes cytotoxicity in fish hepatocytes and can lead to oxidative stress, resulting hepatotoxic in fish. Thus, the utilization of BPS instead of BPA as safe alternative in industry should be re-evaluated in the future for environmental health.

Keywords Bisphenol S · Oncorhyncus mykiss · Fish hepatocytes · Cytotoxicity · Antioxidant defences

Introduction

It is well-known that bisphenol A (BPA) is a an endocrine-disrupting chemical, as well as a suspected carcinogen that is used in the production of plastic materials, food packaging, medical equipment, dental sealants, baby bottles, adhesives, flame retardants, toys, and thermal receipts [1]. Due to its potential hazardous impacts in humans and risks to wildlife, scientific milieu and regulators have raised concerns regarding use of BPA throughout the world [2]. Consequently, efforts toward restriction and legislation for usage of BPA have been taken by the European Union and United States [3, 4] and safer alternatives have been sought instead of BPA in industrial applications. Thus, manufacturers have been prompted to replace BPA with its structural derivatives, such as bisphenol S (BPS), so as to comply with those regulations [5]. BPS has been frequently utilized as an electroplating solvent, wash-fastening agent, and component of phenolic resin [6]. This analogue is commonly used in daily products, such as epoxy glues, thermal receipts, canned food stuffs, paper currencies, luggage tags, food cartons, and baby bottles [7]. Products in which BPS has been used as a component, such as thermal papers and plastics, are labelled as ‘BPA-free’ in marketing [8]. Environmental monitoring studies have reported the presence of BPS in indoor dust, foodstuff, sediments, surface waters, and sewage sludges.
in different countries [5, 9]. Average concentrations of BPS in the rivers of Japan, Korea, China, and India were reported to be detected from undermined levels, up to 15, 42, 135, and 7200 ng/L, respectively [9]. The estimated daily dietary intake (ng/kg body weight/day) of BPS for infants, toddlers, children, teenagers, and adults, were averagely determined to be 1.72, 4.34, 2.49, 1.60, and 1.31 ng/kg body weight, respectively, in the United States [10]. In the human urine samples that were examined in various countries around the world, BPS concentrations of 1.18 ng/mL, 0.933 µg/g creatinine (Cre) were determined in Japan, as well as 0.299 ng/mL, 0.304 µg/g Cre in the United States, 0.226 ng/mL, 0.223 µg/g Cre in China, 0.172 ng/mL, 0.126 µg/g Cre in Kuwait, and 0.160 ng/mL, 0.148 µg/g Cre in Vietnam [5]. As inhabitants of aquatic environments, fish are inevitably exposed to BPA and its analogues via their surrounding milieu. In a recent study, BPA was detected in the liver and muscle of Trachurus trachurus, Dicentrarchus labrax, and Scomber colias from the North East Atlantic Ocean, as well as bisphenol B and bisphenol E in the muscle [11]. Experimental studies over the last decade have shown that exposure to BPS causes developmental abnormalities, reproductive impairment, and hormonal imbalance in fish. The treatment of zebrafish (Danio rerio) with BPS resulted in a skewed sex ratio towards the side of females, low testosterone levels in males, significant escalations in plasma vitellogenin, decreased levels of plasma thyroxine and triiodothyronine, decreases in egg production and sperm count, and lower hatching rate [12]. BPS has been also reported to possess estrogenic, androgenic, and anti-androgenic activity, and genotoxic impacts [2, 13].

The characterization of oxidative stress includes an imbalance between anti-oxidants and pro-oxidants that can be simulated by xeno-estrogens and antioxidant system indicators, which are useful tools for the determination of harmful effects of a specific pollutant [14]. On the other hand, very scarce information regarding BPS cytotoxicity in fish is available in the literature data.

Fish cells are inexpensive and suitable tools that can be used for testing chemicals in toxicological studies. Therefore, in this study, the isolated and primary cultured hepatocytes of Oncorhyncus mykiss were used, as a result of it being a test animal that was recommended for use in toxicological studies by the Organization for Economic Co-operation and Development [15], to shed light on the harmful effects of BPS at cellular level.

**Materials and methods**

**Fish**

Five juvenile rainbow trout (Oncorhyncus mykiss) (fork length: 20–21.5 cm; total weight: 106–136 g) were used in the study. The fish were obtained from a locally owned rainbow trout farm that was located in Van Province, Turkey.

**Preparation of isolated hepatocytes**

The process of hepatocyte isolation from the fish was performed using the method given in the study of Mortensen et al. [16], with some modifications. First, the livers were aseptically dissected from the fish and put into Petri dishes that contained cold Ca²⁺-free solution-I (7.14 g/L of NaCl, 0.36 g/L of KCl, 0.15 g/L of MgSO₄, 1.6 g/L of Na₂HPO₄, 0.4 g/L of NaH₂HPO₄, 0.31 g/L of NaHCO₃, and 20 mg/L of EGTA). Next, the samples of liver tissue were minced in this solution, and then all of the blood was removed, as well as any remaining rude components of tissue, such as connective tissue and vessels. As a next step, the liver was dissociated mechanically into even smaller pieces via the use of surgical blades and fine forceps. Next, pieces of tissue, which were now whitened, were transferred into solution-II, which possessed the same compounds that were included in solution-I, with the addition of 0.11 g/L of CaCl₂ rather than EGTA, as well as 0.025 mg/mL of type IV collagenase, which was used to break the Ca²⁺-dependent cell-cell connections and enzymatically dissociate the cells. After the pieces of tissue had been softened in solution-II for 10 min, they were pulverized using pipette tips of varying sizes. Next, the crude particles were then removed via the use of a stainless steel sieve and the cell suspension that it yielded was very carefully passed through a sterile injector needle to further dislocate the cells. Then, the suspension was placed into Eppendorf tubes, which were then centrifuged at 60× g for 3 min. The cell pellet was then suspended in Leibovitz (L-15) medium containing 0.38 g/L of NaHCO₃ and 1% (v/v) antibiotic-antimycotic. After it had been washed for the second time with L-15, the cells were re-suspended in medium. After this, the cells were counted by using a Thoma slide, and the cell viability was assessed using the Trypan blue exclusion test. In the examination of the cell culture, the cells were observed to determine if they possessed >90% viability.

**Hepatocyte culture and BPS treatment**

The isolated hepatocytes were seeded into 24-well culture plates that were coated with collagen-I (Gibco, Catalogue Number: A11428-02, Thermo Fisher Scientific Inc., Waltham, MA, USA) in 1 mL of medium with a cell density of 2×10⁶ per each well. Cells were maintained at a temperature of 14 °C for 48 h in an incubator under sterile conditions (Binder GmbH, Tuttingen, Germany) with atmospheric air and saturated humidity prior to treatment with BPS. A 0.1 M stock solution of BPS (C₁₂H₁₀O₄S; MW: 250.27; 99.7%, Acros Organics, Catalogue Number: 146915000)
was prepared in absolute ethanol and exposure media that contained 15.63, 31.25, 62.50, 125, 250, and 500 µM concentrations of BPS, which were then applied to the cells for a period of 24 h. The doses of BPS in the culture medium were selected according to the previously published in vitro studies [17, 18]. Care was taken to ensure that the final ethanol concentration in the media was not greater than 0.5%. In the control group cells were treated with L-15 medium that only contained 0.5% absolute ethanol. Five replicate wells were used to check the cell cultures.

Lactate dehydrogenase cytotoxicity test

The culture media was examined to determine the amount of lactate dehydrogenase (LDH) leakage that had occurred, and this measurement was then used in the determination of the cytotoxic effects that resulted from the BPS following treatment for 24 h. After the treatments had been completed, culture media were removed from the wells and transferred into Eppendorf tubes. Following this, 10 µL of the samples were put into a 96-well plate and an assay kit to determine LDH cytotoxicity (Catalog No: ab65393, Abcam, Cambridge, UK) was then used to detect LDH activity, following the manufacturer’s instructions. As a final step, a DAS A3 bridge, UK) was then used to detect LDH activity, following the manufacturer’s instructions. The contents of the reaction medium for the GST activity was performed according to Habig and co-workers [20]. Optimization was performed for this using the ELISA Microplate Reader System, which was used on a Multiskan FC microplate photometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) [21]. The contents of the reaction medium for the GST reaction were: 0.1 M of phosphate buffer at a pH of 7.4, 1 mM of 1-chloro-2,4-dinitrobenzene, and 1 mM of GSH. The reaction started through the addition of 25 µL of the sample, which comprised 33.28–73.4 µg per well, and the absorbance data were obtained at 25 °C, for 10 min, at 340 nm.

Measurement of antioxidant defense indicators

After the exposures had been completed, the culture media very carefully pipetted out of the wells and the hepatocytes were then rinsed in 1 mL of ice-cold phosphate buffered saline that had a pH of 7.4. Then, the obtained 2 × 10⁶/mL cell suspensions were transferred into Eppendorf tubes, which were then lysed in a glass-porcelain ultrasonic homogenizer (Jencons Scientific Co., Herts, UK). Next, the homogenate was then centrifuged at 15,000×g for 15 min. All of these procedures were either conducted at 4 °C or on ice. As the next step, the supernatant fractions were taken out and then used to determine the antioxidant defense indicators. In each of the experiments, 5 replicates were conducted.

Spectrophotometric measurement of the SOD activity was conducted with a commercial kit (Ransod, Randox Lab., Crumlin, County Antrim, UK) by following the manufacturer’s instructions. The method listed in the kit is consisted of a xanthine-xanthine oxidase system that was utilized in the generation of superoxide radicals, which reacted with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) and resulted in the formation of red formazan dye. Measurement of the SOD activity was then conducted using the degree of inhibition determined from this reaction. It was determined that 1 unit of SOD was the amount to result in 50% inhibition of the reduction rate of INT under the conditions that were given in the assay. All of the measurements were conducted at 37 °C and 505 nm, and the activity was expressed as units/gram protein.

Determination of glutathione S-transferase (GST) activity was performed according to Habig and co-workers [20]. Optimization was performed for this using the ELISA Microplate Reader System, which was used on a Multiskan FC microplate photometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) [21]. The contents of the reaction medium for the GST activity measurements were 0.1 M of phosphate buffer at a pH of 7.4, 1 mM of 1-chloro-2,4-dinitrobenzene, and 1 mM of GSH. The reaction started through the addition of 25 µL of the sample, which comprised 33.28–73.4 µg per well, and the absorbance data were obtained at 25 °C, for 10 min, at 340 nm.

The contents of the reaction medium for the GST Theta 1-1 (GSTT1-1) isozyme activity measurements were 0.1 M of phosphate buffer at pH 6.5, 0.25 mM of...
1,2-epoxy-3-(p-nitrophenoxy)-propane, and 0.5 mM of GSH. The reaction was started through the addition of 100 µL of the sample, which comprised 133.2–293.6 µg per well, and the absorbance data were obtained at 360 nm, for 10 min, at 25 °C [21]. Specific activity was calculated by using Eq. (2):

\[
\text{Specific activity} = \frac{\Delta A/\Delta t}{\epsilon \text{(mM}^{-1}\text{cm}^{-1})} \times DF \times \frac{1}{\text{mg of protein/mL}}
\]  

(2)

Here, \(\Delta A/\Delta t\) is the absorbance change per minute, \(\epsilon\) is the extinction coefficient of the substrates (9.6 mM\(^{-1}\) cm\(^{-1}\) for CDNB and 0.5 mM\(^{-1}\) cm\(^{-1}\) for EPNP), and DF is the dilution factor.

Determination of the total thiol content was performed following the method described by Sedlak and Lindsay [22], which had been optimized for the ELISA Microplate Reader System. The standard curve was built with the measurement results of the GSH standards in the concentration range of 0.1–1 mM. Next, 50 µL of sample, comprising 66.6–146.8 µg/well, was added to 30 µL of 0.2 M Tris buffer at a pH of 8.2. Right after that, 20 µL of 2 mM 5,5′-Dithiobis-(2-Nitrobenzoic acid) (DTNB) and 100 µL of methyl alcohol were added into each well. The plate was then incubated, under dark conditions, for 30 min at 25 °C. The absorbance was measured at 405 nm. Calculation of the total amount of thiol within the samples was performed by using the slope value of the standard curve, the results of which were expressed as nmole/mg of protein.

The content of malondialdehyde (MDA), which is a product of lipid peroxidation, was spectrophotometrically measured following the method of Beuge and Aust [23]. Briefly, 0.1 mL of 150 mM Tris-HCl buffer, at pH 7.1, was added on the supernatant (0.1 mL). Next, 0.1 mL of 1.5 mM ascorbic acid and 0.1 mL 1 mM FeSO\(_4\) were added to this mixture. The total volume of this mixture was completed to 1 mL by the addition of distilled water and then the tubes were incubated for 15 min at 37 °C. As a next step, 1 mL of trichloracetic acid (10%) and 2 mL of thiobarbituric acid (0.375%) were added to the mixture, and the tubes were incubated in boiling hot water for 15 min. After the tubes had been cooled, centrifugation was performed at 1000 g for 10 min. Spectrophotometric measurements were carried out at 532 nm. Calculation of the concentration of MDA in samples was performed using the standard curve that was derived from external 1,1,3,3-tetraethoxypropane standards. The results were expressed as nmole/mg of protein.

Spectrophotometric assay of the total protein content within the supernatant fractions was performed using the method published by Bradford [24], using bovine serum albumin as the standard.

**Fig. 1** Photomicrographs showing the rainbow trout cultured hepatocytes. a healthy control cells clustering themselves into groups. b Cells that were exposed to a BPS concentration of 500 µM for 24 h, which display degenerative changes (shown with arrows)

### Statistical analyses

All of the statistical analyses were conducted using IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). One-way ANOVA and the Duncan multiple comparison post-hoc test were used to analyze differences among the groups. The results were expressed as a mean ± standard error. p < 0.05 was considered statistically significant.

### Results

After the experiments had been completed, microscopic examination of the control group hepatocytes exhibited a healthy appearance (Fig. 1a). The hepatocytes that has been treated exhibited degenerative changes, such as apoptotic figures or shrinkage, and they did not show healthy appearance as the cells in the control group (Fig. 1b).
As a result of the treatment applied in this study, it was observed that all of the BPS concentrations resulted in a significant increase LDH leakage into the media. The percentage of cellular cytotoxicity increased gradually with the increasing BPS concentrations (p < 0.05). Cytotoxicity occurred along with BPS concentrations of 1.34 ± 0.31, 7.23 ± 0.15, 8.61 ± 0.30, 10.17 ± 0.81, 13.71 ± 0.67, and 18.00 ± 1.18% with 15.63, 31.25, 62.50, 125, 250, and 500 µM, respectively (Fig. 2a). All of the increased levels of cytotoxicity resulted to be statistically significant, except for the 15.63 µM concentration of BPS.

The hepatocyte SOD activity exhibited a significant decrease (p < 0.05; Fig. 2b), whereas the CAT activity significantly increased (p < 0.05; Fig. 2c). The GPx activity was significantly increased with all of the BPS concentrations (Fig. 2d; p < 0.05). GST specific activity was also detected to be elevated (p < 0.05) along with the increasing amount of BPS (Fig. 2e). On the contrary, specific activity of GSTT1-1 isozyme in the samples, except the one that had the lowest BPS concentration, of 15.63 µM, were all lower than (p < 0.05) those that were in the control group (Fig. 2f). An alteration similar to the change in the GSTT1-1 isozyme was observed in the amount of total thiol groups considered as an indicator of the GSH pool of the cell. It was determined that the GSH pool shrunk (p < 0.05) in all the experimental groups in comparison with the control group, except for the one that was treated with the lowest BPS concentration (Fig. 3a). The MDA content remained unchanged with 15.63, 31.25, and 62.50 µM concentrations of BPS, while it displayed statistically significantly increases with 125, 250, and 500 µM concentrations of BPS (Fig. 3b).

Fig. 2 Graphs depicting the cytotoxic effects of the varying BPS concentrations on the rainbow trout cultured hepatocytes following exposure for 24 h (a), and the in vitro effects of the varying BPS concentrations on the LDH leakage into the medium (a), SOD (b), CAT (c), GPx (d), GST (e), and GSTT1-1 (f) specific activities. The different letters indicate the statistically significant differences that were observed between the treatments.
Discussion

The percentage of hepatocyte cytotoxicity gradually increased after the BPS treatment according to the LDH cytotoxicity test. Those increases were statistically significant with concentrations of 31.25 µM or higher, and it was determined that the percentage of cytotoxicity was 17.01% following treatment with the highest concentration of BPS. Similar results were also reported in several studies using different techniques, wherein BPS induced cytotoxicity or decreased cell viability in different cell types. For example, Hercog et al. [25] found that BPS decreased cell viability in human hepatocellular carcinoma cells with a BPS concentration of 20 µg/mL after 72 h exposure. In a study by Kose et al. [18], cell viability gradually descended in RWPE-1 cells after exposure to BPS for 24 h with concentrations ranging from 0 to 600 µM, and they determined that the inhibitory concentration 20 (IC20) and 50 (IC50) for BPS was 108 µM and 380.90 µM, respectively. The percentage of cell viability decreased to 61.30% in TM3 Leydig cells that had been treated with a 50 µg/mL concentration of BPS for 24 h [26]. These different results, even at similar concentrations, among studies on BPS-induced cytotoxicity might be related to cell type, experimental design of the study, duration of treatment used, and the assays that were selected in the determination of cytotoxicity. Supporting, Russo et al. [27] reported that different cell types displayed different sensitivities to BPA and its analogues, and that the 48-h inhibitory concentrations of 50 µg/mL of BPS for 3T3-L1, MCF-7, C6, and HeLa cells were >100 µM, >100 µM, 168.4 µM, and 299.3 µM, respectively.

SOD and CAT constitute the first barrier for the elimination of superoxide radicals, as well as hydrogen peroxide, in the antioxidant system. As a free radical scavenging enzyme, SOD is responsible for the dismutation of highly-reactive and toxic superoxide anions into molecular oxygen and hydrogen peroxide. The current results showed that all of the concentration of BPS led to general decreases in SOD activity. In accordance with the results obtained herein, lower SOD activity were determined in the reproductive tissues of male rat offspring that had been exposed to BPA and its analogues including BPS. The levels of SOD activity were also reduced in human red blood cells treated with BPA, BPAF, and BPB for 4 and 24 h, whereas BPS did not create significant changes in the SOD activity [28]. Kose et al. [18] also reported diminished SOD activity in RWPE-1 cells that had been incubated with BPA and BPB for 24 h, except for BPS. The differences between the studies may have arisen from the susceptibility of the cell types to BPS or the severity of the oxidative stress provoked by BPS. A probable reason for deceased SOD activity in this study might have arisen from the excessive production of superoxide anions as a result BPS exposure, as the substrate of the enzyme might have behaved as an oxidant [29]. SOD is susceptible to oxidation and it was demonstrated that the enzyme was inactivated by hydrogen peroxide [30]. Dimitrova et al. [31] reported that superoxide radicals or hydrogen peroxide generated via superoxide radical transformation might have caused oxidation of the cysteine in the enzyme that resulted in decreased SOD activity. Thus, increased levels of superoxide anions or excessive hydrogen peroxide levels in the hepatocytes after BPS exposure might have caused such an effect in the current study. CAT, which is an essential antioxidant enzyme for cells, degrades hydrogen peroxide into H₂O and oxygen. The generating hydrogen peroxide induces the activity of the enzyme. It was reported that CAT activity was elevated in mouse liver and renal cells after incubation for 12 h with BPS depending on the ROS production in those cells. In addition, BPS is capable of interacting with the enzyme via binding to the Gly 117 residue on the substrate channel, thus affecting

![Graphs depicting the in vitro effects of the varying BPS concentrations on the GSH (a) and MDA (b) contents. The different letters indicate the statistically significant differences that were observed between the treatments](image)
ever, elevated GSH levels were found in RWPE-1 cells that were exposed to BPS. However, significant decreases in the GSH content were reported in zebrafish embryos after a short time exposure to BPA and its derivatives, like BPS. Similar results were also reported as elevated GST specific activity in the liver tissues of freshwater species like male fathead minnow (Pimephales promelas) and Japanese medaka (Oryzias latipes). In the current study, BPS treatment of Oncorhyncus mykiss hepatocyte culture unveiled the same response of elevated total GST specific activity, demonstrating the practicality of the usage of this parameter in the testing of damage of xenobiotic exposure to aquatic animals.

GST Theta is known as the first isozyme evolved among others, which consists of two different types: GSTT1 and GSTT2, sharing 55% protein sequence identity, and, to date, it has been subjected to characterization in several aquatic organisms, such as Macrobrachium rosenbergii, Ruditapes philippinarum, and Apostichopus japonicus. Although its major sites of expression are the gills and epithelium in the olfactory and digestive systems of rainbow trout, weak but debatable specific activity of GSTT1-1 was measured in the liver hepatocyte culture for the first time in this current study. GST Theta types have been considered to play a role in xenobiotic biodegradation, even though their substrate specificities are different from each other, which might explain the decrease in the measured specific activity with the increasing BPS concentration in the media, while the total GST activity was scaled up in the treatment groups.

OXIDANTS, INCLUDING RADICALS AND ROS, MAY ATTACK CARBON-CARBON DOUBLE-BOND-CONTAINING LIPIDS, SUCH AS
polyunsaturated fatty acids in biological membranes. During the oxidation of unsaturated fatty acids, a range of changes, including hydrogen removal from a carbon and the insertion of oxygen instead of it, occurs in target lipids involving glycolipids, phospholipids, and cholesterol. Finally, lipid peroxyl radicals as well as hydroperoxides cause an impairment in membrane function and leads to apoptosis in cells [46]. Among lipid peroxidation products, MDA is the most mutagenic and it has been widely used as a biomarker, which reflects indirectly the lipid peroxidation levels following free radical attacks in cells [47]. The findings determined in the current research displayed clearly that there was a significant elevation in the MDA content after the exposure to 125 µM or higher concentrations of BPS, and such increases resulted to be inversely correlated with decreased GSH levels. Similar to the results herein, rat spermatozoa displayed high ROS and thiobarbituric acid reactive substances (TBARS) levels after incubation with BPS [48]. In another study, the incubation of rat testicular tissue with BPS for 2 h caused the formation of ROS as well as an increase in lipid peroxidation level in vitro [49]. Ullah et al. [50] found increased lipid peroxidation levels quantified by TBARS in the reproductive tissues of the male offspring of Sprague Dawley rats that were exposed to BPA, and its analogues, with concomitant increases in the ROS levels, indicating that the accumulation of ROS could attack unsaturated fatty acids, and finally, lead to lipid peroxidation as the result of BPS. On the other hand, Maćczak et al. [28] stated that BPA and its analogues, BPAF, BPF, and BPS, induced ROS levels in red blood cells, whereas BPS did not cause increased lipid peroxidation, as was induced by BPA and the other analogues. Russo et al. [39] reported that the cytotoxicity of bisphenol analogues was slightly related with phospholipophilicity in different cell lines. Thus, it can be concluded that significant increases in the MDA content observed in this study might have arisen from the elevated levels of ROS, caused by BPS, which lead to accelerated lipid peroxidation.

In summary, the present research is the first report which demonstrates the induction of toxic effects by BPS on isolated rainbow trout hepatocytes. BPS, used as a substitute instead of BPA as an alternative in the food packaging or other application areas of industry, changed the levels of antioxidant defense enzymes, namely SOD, CAT, GPx, GST, and GSTT1, decreased the GSH content, and caused lipid peroxidation in the cells. The results provided evidences that BPS is not a safer, innocent agent that can be used as an alternative to BPA. Authorities should reconsider the utilization of BPS in the industry and take measures to prevent environmental contamination of this compound, which may affect the antioxidant system in organisms.

**Author Contributions** This research was conceived by BK. The isolation and culturing of the hepatocytes, and the application of the treatments were performed by BK, HA, ED, MB and FY. The cytotoxicity testing was performed by BK, HA, ED, and FY. The antioxidant defense system indicators were measured by BK, CY, HA, ED, CF, FY, and MB. Analyzing of the data and interpreting the results were performed by BK and CY. The drafting and editing of the manuscript were performed by BK and CY. The manuscript was finalized by BK.

**Funding** The authors declare that no financial support was received for either the research or the writing of this article.

**Data availability** All of the data and the material that were used and analyzed in the course of the study herein can be obtained from the author for correspondence upon reasonable request.

**Declarations**

**Conflict of interest** The authors declare that they have no conflicts of interest with regards to this research.

**Ethical approval** The procedures that were conducted within this research were all performed in line with the procedures set forth by the National and Institutional Regulations for the Protection of Animal Welfare. The necessary permissions were obtained from the Ethical Committee of the Animal Experiments Ethics Committee of Van Yuzuncu Yil University, under decision No.: 2021/03-15 and protocol No.: E.37149.

**References**

1. Seachrist DD, Bonk KW, Ho SM, Prins GS, Soto AM, Keri RA (2016) A review of the carcinogenic potential of bisphenol A. Reprod Toxicol 59:167–182. https://doi.org/10.1016/j.reprotox.2015.09.006
2. Rochester JR, Bolden AL (2015) Bisphenol S and F: a systematic review and comparison of the hormonal activity of bisphenol A substitutes. Environ Health Perspect 123(7):643–650. https://doi.org/10.1289/ehp.1408989
3. European Union (2011) Amending Directive 2002/72/EC as Regards the Restriction of Use of Bisphenol a in Plastic Infant Feeding Bottles, Commission Directive 2011/8/EU of 28 January 2011
4. US Food and Drug Administration (2013) Amended the regulation 21 CFR175 as regards no longer use bisphenol a in the coating of packaging for powdered and liquid infant formula
5. Liao C, Liu F, Alomirah H, Loi VD, Mohd MA, Moon H al (2012) Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures. Environ Sci Technol 46:6860–6866
6. Clark E (2012) Sulfolane and sulfones. Kirk-Othmer encyclopedia of chemical technology, Wiley, New York
7. Liao C, Li F, Kannan K (2012) Bisphenol S, a new bisphenol analogue, in paper products and currency bills and its association with bisphenol a residues. Environ Sci Technol 46:6515–6522
8. Atlas E, Dimitrova V (2019) Bisphenol S and bisphenol A disrupt morphogenesis of MCF-12A human mammary epithelial cells. Sci Rep 9(1):1–10
9. Yamazaki E, Yamashita N, Taniyasu S, Lam J, Lam PK, Moon H al (2015) Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India. Ecotoxicol Environ Saf 122:565–572
10. Liao CY, Kannan K (2013) Concentrations and Profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for Human Exposure. J Agric Food Chem 61(19):4655–4662

11. Barboza LGA, Cunha SC, Monteiro C, Fernandes JO, Guilhermino L (2020) Bisphenol A and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers. J Hazard Mater 393:22419. https://doi.org/10.1016/j.jhazmat.2020.122419

12. Naderi M, Wong MY, Gholami F (2014) Developmental exposure of zebrafish (Danio rerio) to bisphenol-S impairs subsequent reproduction potential and hormonal balance in adults. Aquat Toxicol 148:195–203

13. Frenzilli G, Martorell-Ribera J, Bernardeschi M, Scarcelli V, Jöns E, Diano N, Moggio M, Guidi P, Sturke J, Asker N (2021) Bisphenol A and bisphenol S induce endocrine and chromosomal alterations in brown trout. Front Endocrinol 12:645519

14. Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M (2006) Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol Environ Saf 64(2):178–189

15. OECD (2019) Test no. 203: fish, acute toxicity test. OECD guidelines for the testing of chemicals, Sect. 2. OECD Publishing, Paris. https://doi.org/10.1787/9789264069961-en

16. Mortensen AS, Tolfsen CC, Aruwea A (2006) Gene expression patterns in estrogen (nonylphenol) and aryl hydrocarbon receptor agonists (PCB-77) interaction using rainbow trout (Oncorhynchus mykiss) primary hepatocyte culture. J Toxicol Environ Health 69(1-2):1–19. https://doi.org/10.1080/15287390500257792

17. Feng Y, Jiao Z, Shi J, Li M, Guo Q, Shao B (2016) Effects of bisphenol analogues on steroidogenic gene expression and hormone synthesis in H295R cells. Chemosphere 147:9–19. https://doi.org/10.1016/j.chemosphere.2015.12.081

18. Kose O, Rachidi W, Beal D, Erkekoglu P, Fayyad-Kazan H, Kocer N (2019) Assessment of the effective impact of bisphenols on mitochondrial activity and steroidogenesis in a dose-dependency in mice TM3 Leydig cells. Physiol Res 68(4):689–693. https://doi.org/10.33549/physiologes.934200

19. Russo G, Capuozzo A, Barbato F, Irace C, Santamaria R, Grumetto L (2018) Cytotoxicity of seven bisphenol analogues compared to bisphenol A and relationships with membrane affinity data. Chemosphere 201:432–440. https://doi.org/10.1016/j.chemosphere.2018.03.014

20. Maćzak A, Cyrkler M, Bukowska B, Michałowicz M, Piontek E, Diano N, Moggio M, Guidi P, Sturke J, Asker N (2021) Bisphenol A, bisphenol S, bisphenol F and bisphenol AF induce different oxidative stress and damage in human red blood cells (in vitro study). Toxicol In Vitro 41:143–149. https://doi.org/10.1016/j.tiv.2017.02.018

21. Modesto KA, Martinez CB (2010) Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish Prochilodus lineatus. Chemosphere 78:294–299. https://doi.org/10.1016/j.chemosphere.2009.10.047

22. Pigolet E, Corbissier P, Aubin A, Lambert D, Michieles C, Raes M, Zachary MD, Remacle J (1990) Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. Mech Ageing Dev 51(3):283–297. https://doi.org/10.1016/0047-6374(90)90078-t

23. Dimitrova MST, Tisnova V, Velcheva V (1994) Combined effect of zinc and lead on the hepatic superoxide dismutase-catalase system in carp (Cyprinus carpio). Biochem Physiol C Toxicol 148:103–46

24. Zhang R, Liu R, Zong W (2016) Bisphenol S interacts with catalase and induces oxidative stress in mouse liver and renal cells. J Agric Food Chem 64(34):6630–6640

25. Monteiro DA, Rantin FT, Kalinin AL (2009) The effects of selenium on oxidative stress biomarkers in the freshwater characid fish matrix. Brevyn cephalus exposed to organophosphate insecticide FoliSuper 600 (methyl parathion). Biochem Physiol C Toxicol 149(1):40–49

26. Aykut H, Kaptaner B (2021) In vitro effects of bisphenol F on antioxidant system indicators in the isolated hepatocytes of rainbow trout (Oncorhyncus mykiss). Mol Biol Rep 48:2591–2599. https://doi.org/10.1007/s11033-021-06310-3

27. Meister A, Anderson ME (1983) Glutathione. Annu Rev Biochem 52(1):711–760

28. Wu M, Xu H, Shen Y, Qiu W, Yang M (2011) Oxidative stress in zebrafish embryos induced by short-term exposure to bisphenol A, nonylphenol, and their mixture. Environ Toxicol Chem 30(10):2335–2341

29. Stephensen E, Sturve J, Forlin L (2002) Effects of redox cycling compounds on glutathione content and activity of glutathione-related enzymes in rainbow trout liver. Comp Biochem Physiol C Toxicol 133(3):435–442. https://doi.org/10.1016/s1532-0456(02)00129-1

30. Eroglu A, Dogan Z, Kanak EG, Atli G, Canli M (2015) Effects of heavy metals (Cd, Cu, Cr, Pb, Zn) on fish glutathione metabolism. Environ Sci Pollut Res 22(5):3229–3237

31. Jung JH, Moon YS, Kim BM, Lee YM, Kim M, Rhee JS (2018) Comparative analysis of distinctive transcriptome profiles with biochemical evidence in bisphenol S- and benzo[a]pyrene-exposed liver tissues of the olive flounder Paralichthys olivaceus. PloS One 13(5):e0196425. https://doi.org/10.1371/journal.pone.0196425

32. Yu FT, Rhee JS, Raisuddin S, Lee JS (2008) Characterization of the glutathione S-transferase-Mu (GSTM) gene sequence and its expression in the hermaphroditic fish, Kryptolebias marmoratus as a function of development, gender type and chemical exposure. Chem Biol Interact 174(2):118–125. https://doi.org/10.1016/j.cbii.2008.05.011

33. Zare A, Henry D, Chua G, Gordon P, Habibi HR (2018) Differential hepatic gene expression profile of male fathead minnows exposed to daily varying dose of environmental contaminants.
42. Li D, Chen Q, Cao J, Chen H, Li L, Cedergreen N et al (2016) The chronic effects of lignin-derived bisphenol and bisphenol A in Japanese medaka Oryzias latipes. Aquat Toxicol 170:199–207. https://doi.org/10.1016/j.aquatox.2015.10.024

43. Sandamalika WMG, Priyathilaka TT, Lee S, Yang H, Lee J (2019) Immune and xenobiotic responses of glutathione S-Transferase theta (GST-θ) from marine invertebrate disk abalone (Haliotis discus discus): With molecular characterization and functional analysis. Fish Shellfish Immunol 91:159–171. https://doi.org/10.1016/j.fsi.2019.04.004

44. Pérez-López M, Anglade P, Bec-Ferté M, Debrouwer L, Perdu E, Cravedi JP, Rouimi P (2000) Characterization of hepatic and extrahepatic glutathione S-transferases in rainbow trout (Oncorhynchus mykiss) and their induction by 3,3′,4,4′-tetrachlorobiphenyl. Fish Physiol Biochem 22:21–32. https://doi.org/10.1023/A:1007885332573

45. Coggan M, Flanagan JU, Parker MW, Vichai V, Pearson WR, Board PG (2002) Identification and characterization of GSTT3, a third murine Theta class glutathione transferase. Biochem J 366(1):323–332. https://doi.org/10.1042/bj20011878

46. Yin H, Xu L, Porter NA (2011) Free radical lipid peroxidation: mechanisms and analysis. Chem Rev 111(10):944–5972

47. Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. https://doi.org/10.1155/2014/360438

48. Ullah H, Ambreen A, Ahsan N, Jahan S (2017) Bisphenol S induces oxidative stress and DNA damage in rat spermatocytes in vitro and disrupts daily sperm production in vivo. Toxicol Environ Chem 99(5-6):953–965

49. Ullah H, Jaha S, Ain QU, Shaheen G, Ahsan N (2016) Effect of bisphenol S exposure on male reproductive system of rats: A histological and biochemical study. Chemosphere 152:383–391

50. Ullah A, Pirzada M, Afzar T, Razak S, Almajwal A, Jahan S (2019) Effect of bisphenol F, an analog of bisphenol A, on the reproductive functions of male rats. Environ Health Prev Med 24(1):41. https://doi.org/10.1186/s12199-019-0797-5

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.