Int J Adv Life Sci Res. Volume 8(3) 112-120
https://doi.org/10.31632/ijalsr.2025.v08i03.010

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org

Original Article

Comparing the Toxicity Effect Induced by Bisphenol A and Bisphenol S in the Zebrafish Model

Muhamad Fikri Shazlan Saad[1], Muhammad Nazrul Hakim Abdullah[2], Vuanghao Lim[3], Yoke Keong Yong[1*]

[1]Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang Selangor, Malaysia

[2]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang Selangor, Malaysia

[3]Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam 13200, Kepala Batas, Penang, Malaysia

**Corresponding Author's Email: yoke_keong@upm.edu.my*

Abstract

Introduction: Bisphenol S (BPS) is structurally similar to bisphenol A (BPA), and with the shift from BPA to BPS in consumer products, knowledge of BPS's impact on human health remains limited. This study compares the toxicity of BPA and BPS in zebrafish embryos. The zebrafish embryo model, with conserved vertebrate cardiac development and high sensitivity to environmental toxins, provides an ideal platform to evaluate the comparative safety of BPS. **Methods:** Zebrafish embryos were exposed to BPA and BPS at concentrations of 10 µM and 20 µM in 24-well plates. Mortality rates were assessed at 120 hours post-fertilisation (hpf). Hatching rates were evaluated at 72 and 96 hpf using a modified protocol. Cardiotoxicity was assessed by measuring heart rate and pericardial oedema at 96 hpf. **Results and Discussion**: Neither BPA nor BPS had a significant effect on mortality or hatching rates. In the cardiotoxicity assay, exposure to 10 µM BPA increased the heart rate, while 20 µM BPA significantly ($p<0.05$) reduced it. Both BPS concentrations did not significantly ($p>0.05$) affect the heart rate compared to the control. Pericardial oedema was observed in both BPA-treated groups (10 µM and 20 µM) but not in the BPS-treated groups. **Conclusion:** BPS exhibits lower toxicity than BPA with respect to mortality and cardiotoxicity in zebrafish embryos, although neither compound affected hatching rates.

Keywords: *Cardiotoxicity; Hatching Rate; Mortality*

Introduction

Bisphenols, common byproducts of plastics, are widely used in food containers, milk powder packaging, baby bottles, and dental products. Heating, chemical reactions, or repeated use can lead to bisphenol depolymerization, releasing monomers that may migrate into food and drinks (Khalili Sadrabad *et al*., 2023). Since the 1930s, Bisphenol A (BPA) has been recognised for its estrogenic properties, functioning as an Endocrine Disrupting Compound (EDC) by mimicking estrogen and binding to estrogen receptors, thereby disrupting normal hormonal regulation. BPA acts as both an agonist and antagonist of thyroid receptors and can inhibit endogenous androgen and estrogen activity (Yuan *et al*., 2023; Park *et al*., 2024). This dysregulation can adversely affect the reproductive, cardiovascular, and nervous systems; promote DNA mutations; induce oxidative stress; and contribute to obesity (Kang, Asai & Toita, 2023; García, Picó & Morales-Suárez-Varela, 2024).

Received on :16th January 2025; Revised version received on :30th June 2025; Accepted: 4th July 2025

Growing concerns over BPA's health effects have led manufacturers and policymakers to adopt "BPA-free" alternatives, including Bisphenol F (BPF), Bisphenol AF (BPAF), and Bisphenol S (BPS). BPS, or bis(4-hydroxyphenyl) sulfone, is a widely used BPA substitute with a similar chemical structure, photo resistance, and greater thermal stability (Wu *et al*., 2018). BPS is commonly found in cleaning agents, electroplating solvents, phenolic resins, thermal paper, and various other consumer products, resulting in exposure for over half the population in the U.S. and Asia via diet, drinking water, and household items (Bousoumah *et al*., 2021). Like BPA, BPS exhibits estrogenic activity by interacting with both Estrogen Receptor Alpha (ERα) and Beta (ERβ), and has been associated with obesity and intrahepatic fat accumulation (Alharbi *et al*., 2022; Oliviero *et al*., 2022). Despite its increasing use, the full impact of BPS on human health remains unclear (Yin *et al*., 2019), highlighting the need to assess the effects of low-dose BPA and BPS exposure to better understand potential risks.

Zebrafish embryos are a practical model for toxicological research due to their short life cycle, approximately 70% genetic similarity to humans, and cost-effectiveness (Gautam *et al*., 2024). This study aims to compare the toxic effects of BPA and BPS on zebrafish embryos, with a focus on mortality, hatching rates, heart rate, and pericardial oedema formation.

Materials and Methods

Chemical and Reagents

A 60× E3 media stock solution was prepared by dissolving 17.4 g of Sodium Chloride (NaCl), 0.8g of Potassium Chloride (KCl), 2.9 g of Calcium Chloride Dihydrate (CaCl2. 2H2O), and 4.89 g of magnesium chloride hexahydrate (MgCl2. 6H2O) in 1liter of autoclaved distilled water. The pH was then adjusted to 7.2 using Sodium Hydroxide (NaOH) and verified with a calibrated pH meter. A 1× working E3 media was prepared by diluting 16.5 ml of 60× stock solution into 983.5 mL of distilled water. For this study, 10% Ethanol (EtOH) was used as a positive control in zebrafish embryos. A 0.025 % Dimethyl Sulfoxide (DMSO) was used to dissolve both BPA and BPS, serving as solvent control.

Preparation of Bisphenol A and Bisphenol S

Bisphenol A (BPA) and Bisphenol S (BPS) compounds were purchased from Tokyo Chemical Industry (Tokyo, Japan). Dimethyl Sulfoxide (DMSO) from ATCC was used to dissolve both compounds to a final stock concentration of 100 mM, which was subsequently diluted to the desired working concentration for the assays. The final concentration of DMSO in all treatments was maintained below 0.025% to avoid potential DMSO-induced toxicity in the embryos.

Zebrafish Embryos

Wild-type zebrafish embryos were obtained from the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia. Zebrafish embryo experiments (≤120 hpf) complied with OECD 236 and EU Directive 2010/63/EU Article 1.5, which classify this stage as exempt from animal ethics requirements. Upon collection at 3 to 5 hours post-fertilisation (hpf), dead and unfertilised eggs were removed. Embryo viability was assessed based on the presence of egg coagulation, which indicates embryonic death.

Acute Toxicity Test

Briefly, ten dechorionated wild-type zebrafish embryos at 48 hpf were placed in each well of 24-well plates containing 2.5 ml of E3 media. Separate plates were prepared for BPA and BPS, each divided into three groups: 10 µM, 20 µM, and Internal Control (IC). An additional plate included Internal Control (IC), Solvent Control (SC) and Positive Control (PC) groups. Each group conducted with triplicate technical replicates. Cumulative mortality and morphological deformities were assessed at 120 hpf using a standard stereo microscope. Embryo lethality was determined based on apical observations, including egg coagulation, absence of somites formation, lack of heartbeat and failure of tail detachment from the yolk. Teratogenic effects were identified through visible defects in body length, eye development, heart morphology, yolk sac shape, and the presence of scoliosis (Felisbino *et al*., 2023).

Hatching Rate

To evaluate the hatching rates, zebrafish embryos were treated with varying concentrations of BPA and BPS at 24 hours hpf, using the same experimental setup as previously described. Hatching was monitored at 24-hour intervals over the subsequent three days. At 96 hpf, the total number of hatched and viable embryos was recorded, and the hatching rate was calculated using the formula described by Yang *et al*. (2023) which is: hatching rate = (number of hatched embryos/ total number of living embryos) x 100.

Cardiotoxicity and Heartbeat

Cardiac malformation in zebrafish embryos was assessed by evaluating the presence of pericardial edema as an indicator of cardiovascular toxicity induced by BPA and BPS. The experimental setup followed the previously described protocol. At 24 hours hpf, five dechorionated embryos were exposed to various concentrations of BPA and BPS for three consecutive days. At 96 hpf, three embryos were randomly selected from each group for assessment of heart rate and cardiac morphology. Heart rate was measured by counting the number of beats over a 15-second period and multiplying the result by four to obtain Beats Per Minute (BPM), as described by Thiagarajan *et al*. (2019).

Data Analysis

All experiments were performed with triplicate technical replicates and repeated in three independent biological trials. All data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA, followed by Tukey's post hoc test, with IBM SPSS Statistics version 26.0. A *p*-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

Toxicity effect of BPA and BPS on zebrafish embryos by using acute toxicity assay

Both concentrations of BPA and BPS showed no statistically significant effect on the zebrafish embryo mortality compared to the solvent control (Figure 1).

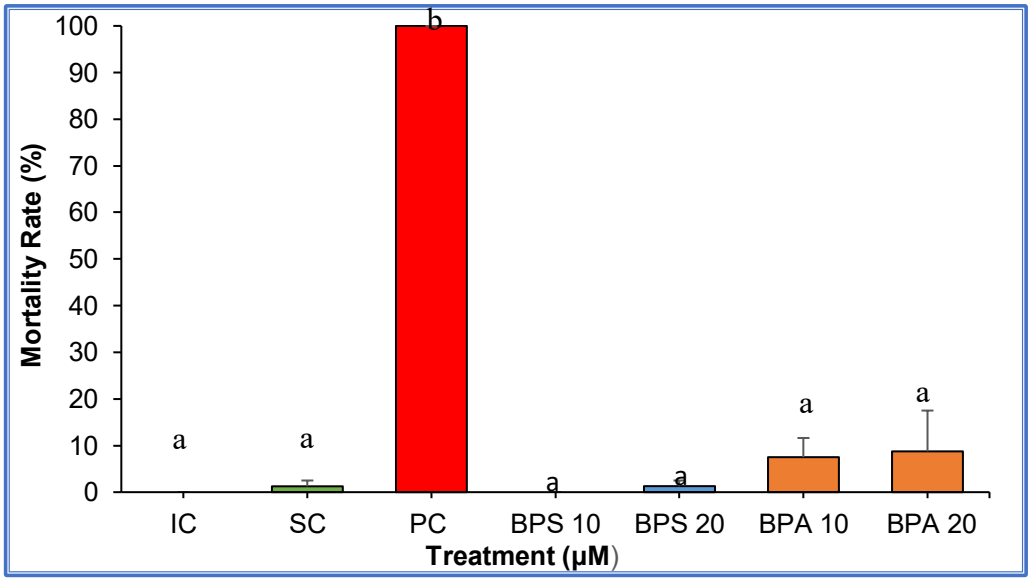


Figure 1: Mortality rates of zebrafish embryos following exposure to varying concentrations of BPA and BPS in comparison with the solvent control. Results are expressed as Mean ± SEM, derived from three independent experiments conducted in triplicate. Abbreviations: IC, internal control; SC, solvent control; PC, positive control; BPS, bisphenol S; BPA, bisphenol A. Differences among the groups were determined by using Tukey's post hoc test ($p<0.05$). Significant differences between groups are represented by different letters, and equal letters indicate no significant difference between treatments.

Exposure to 10 µM and 20 µM of BPS resulted in mortality rates of 0% and 1.25%, respectively. In comparison, BPA caused slightly higher mortality compared to the solvent control (7.5% at 10 µM and 8.75% at 20 µM). Despite these increases, mortality remained low across all groups, and the differences were not statistically significant. Therefore, under the tested conditions (10–20 µM), neither BPA nor BPS induced significant acute lethality in zebrafish embryos.

Effect of BPA and BPS on hatching rate in zebrafish embryos

Hatching rates of zebrafish embryos exposed to BPA and BPS showed no significant difference compared to the solvent control at both 72 and 96 hpf (Figure 2). At 72 hpf, embryos exposed to 10 µM and 20 µM of BPA exhibited hatch rates of 65% and 63.75%, respectively, while those treated with BPS had hatch rates of 68.75% (10 µM) and 78.75% (20 µM). By 96 hpf, hatchability improved across all groups, ranging from 98.75% to 100% indicating that neither compound impaired hatching under the conditions tested.

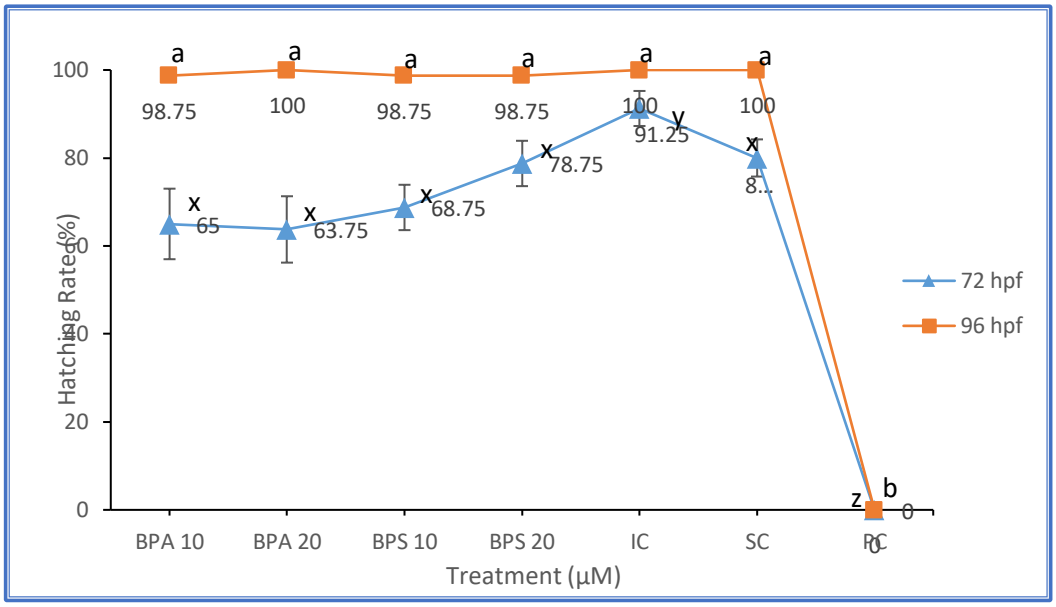
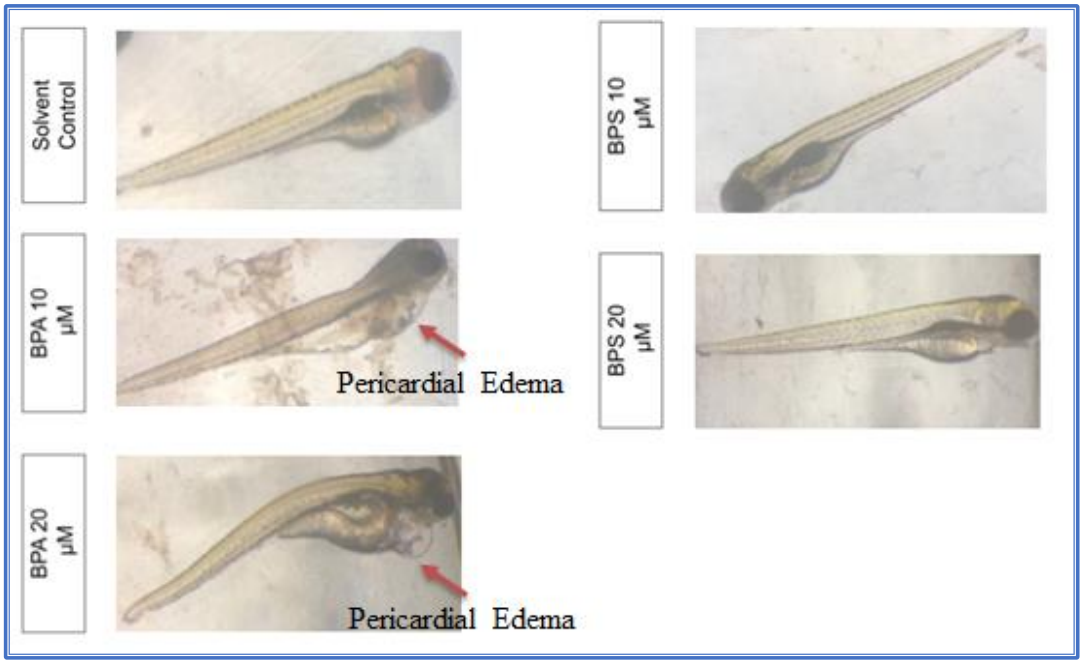


Figure 2: and Figure 1 Hatching rates of zebrafish embryos exposed to BPA and BPS at 72- and 96-hours post-fertilisation (hpf). Treatments: BPA 10 (10 µM BPA), BPA 20 (20 µM BPA), BPS 10 (10 µM BPS), BPS 20 (20 µM BPS), IC (internal control), SC (solvent control), RC (reference control). Data represent mean ± SEM (three independent experiments conducted in triplicate). Different lowercase letters indicate significant differences ($p < 0.05$, one-way ANOVA with Tukey's post hoc test) between treatments at the same time point. Treatments sharing the same letter are not statistically different.

Cardiotoxicity effect of zebrafish larvae treated with BPA and BPS solution

Cardiac assessments (Figure 3A and 3B) revealed that embryos exposed to 10 µM of BPA showed a slightly elevated heart rate, which remained within the normal physiological range. In contrast, 20 µM BPA caused a significant reduction in heart rate ($p < 0.05$). Embryos exposed to BPS at both concentrations displayed normal heart rates, with no significant difference compared to the control group. Additionally, pericardial edema was observed in both BPA-treated groups (10 µM and 20 µM) but was absent in all BPS-treated groups (Figure 3A), suggesting lower cardiotoxicity associated with BPS exposure.

a)



b)

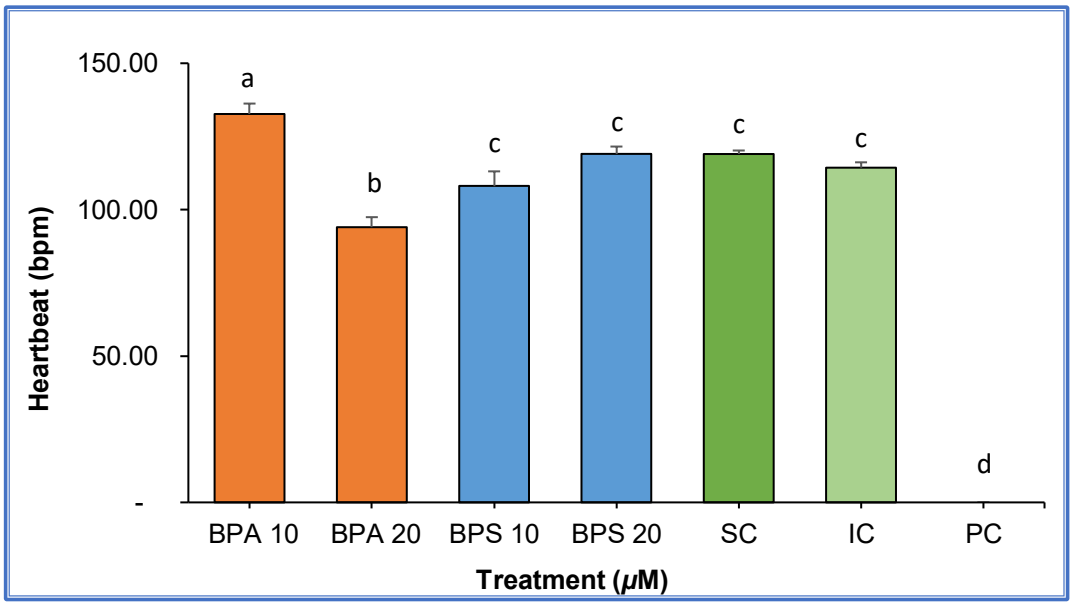


Figure 3: Evaluation of cardiotoxicity in zebrafish embryos after 72 hours of exposure to 10 µM and 20 µM concentrations of BPA and BPS. (a) Pericardial edema observed in zebrafish embryos treated with 10 µM and 20 µM BPA compared to the solvent control. (b) Heart rate (beats per minute) of zebrafish embryos exposed to BPA, BPS, and controls at varying concentrations. Data are presented as mean ± SEM, based on three independent experiments conducted in triplicate. Abbreviations: IC, internal control; SC, solvent control; PC, positive control; BPS, bisphenol S; BPA, bisphenol A. Differences among the groups were determined by using Tukey's post hoc test. Significant differences between groups are represented by different letters, and equal letters indicate no significant difference between treatments.

Discussion

The concentration and duration of chemical exposure are critical factors in assessing environmental toxicology, particularly during early developmental stages when organisms are more vulnerable. Early exposure to toxicants can disrupt normal development in both humans and other organisms, making

zebrafish a valuable model for examining environmental pollutants under controlled early-life-stage conditions. The findings indicate that exposure to 10 and 20 µM BPA did not significantly affect zebrafish embryo survival at 72 hpf, aligning with previous studies reporting no mortality from low-dose BPA (10 µM) at 48 hpf (Karunarathne *et al*., 2021). Similarly, BPS exposure also had no effect on embryo survival, which is consistent with reports showing no mortality at low concentrations, with the LC50 as high as 620 µM (Han *et al*., 2021). Although neither BPA nor BPS caused significant mortality, BPA exposure resulted in a slightly higher mortality rate (7.5% at 10 µM) compared to BPS (1.25% at 20 µM), suggesting that BPS is less toxic to zebrafish embryos. This is in agreement with previous studies indicating low mortality even at higher BPS concentrations (10–200 µM) (Mu *et al*., 2018). BPA has been shown to induce oxidative stress through increased reactive oxygen species (ROS) formation, lipid peroxidation, and altered antioxidant enzyme activity in vitro, whereas BPS does not elicit these toxic responses (Maćczak *et al*., 2017). The observed similarity in the binding modes of BPA and BPS to zebrafish estrogen receptor (zfER) ligands suggests comparable endocrine-disrupting mechanisms (Yin *et al*., 2019). However, BPA's stronger binding affinity to zfER subtypes (zfERα, zfERβ1, zfERβ2) promotes estrogenic disruption by mimicking estradiol, while BPS, with its lower potency, may exert weaker estrogenic effects (Rosenmai *et al*., 2014).

Both BPA and BPS (10 and 20 µM) had no significant impact on hatching rates at 72 and 96 hpf, consistent with findings in *Oryzias melastigma* larvae exposed to 0.8–4.3 µM BPA (Huang *et al*., 2012) and zebrafish embryos exposed to 1 µM BPA or BPS (Gyimah *et al*., 2021). Zebrafish hatching depends on the activity of high and low choriolytic enzymes (HCE and LCE), which degrade the chorion to facilitate emergence (Di Lombo *et al*., 2023). BPA has been demonstrated to have relatively low potency in inhibiting these enzymes compared to other toxicants (Huang *et al*., 2017), although it may still influence early developmental processes. Given the structural similarity between BPA and BPS (Yin *et al*., 2019; Qiu *et al*., 2019), BPS may exert a similar effect on hatching, though likely at higher concentrations.

Exposure to 10 µM BPA increased zebrafish heart rate, in agreement with previous findings at lower concentrations (1 µM) (Gyimah *et al*., 2021; Huang *et al*., 2021). However, the observed increase remained within the normal physiological ranges for zebrafish (120–180 bpm) (Perumal *et al*., 2022). In contrast, exposure to 20 µM BPA significantly reduced heart rate, potentially due to autonomic dysfunction (Belcher, Gear & Kendig, 2015). BPA has been associated with cardiac deformities in zebrafish, possibly through interactions with cardiomyocytes and the alteration of gene expression in developing offspring (Lombó *et al*., 2015). Additionally, BPA induced pericardial oedema (PE) at both 10 and 20 µM, consistent with previous findings where low concentrations (0.3–1 µM) triggered PE at 96 hpf (Gyimah *et al*., 2021; Chen, 2013). BPA's strong binding affinity to zfERα, which in vitro exceeds that of estradiol (Cano-Nicolau *et al*., 2016), may contribute to its cardiovascular toxicity, particularly given zfERα's critical role in heart development (Gorelick *et al*., 2014). Although the molecular mechanisms underlying estrogen signalling in zebrafish cardiac development remain underexplored, zfER ligands are known to modulate heart rate (Pinto *et al*., 2019). Moreover, BPA's affinity for human ERβ, which is expressed in cardiomyocytes, suggests a potential link to disrupted cardiogenesis and arrhythmias via endocrine interference (Lombó *et al*., 2015; Pinto *et al*., 2014). In contrast, BPS exposure did not significantly affect heart rate or induce PE. This reduced cardiotoxicity may be attributed to BPS's lower binding affinity for zfERs, resulting in weaker estrogenic and cardiovascular effects compared to BPA (Mu *et al*., 2018).

Conclusion

The findings indicate that BPS exhibits lower estrogenic effect than BPA in zebrafish embryos, particularly with respect to cardiotoxicity and mortality. While BPA does not appear to inhibit hatching enzyme expression, it may still influence developmental processes. Given BPS's reduced estrogenic potency compared to BPA, it likely poses an equal or lower risk of disrupting the hatching process in zebrafish embryos. Nevertheless, BPS may exert mild estrogenic effects through limited binding to zfER ligands.

Conflict of Interest

The authors declare that they have no competing interest.

Acknowledgement

This study received partial funding from the Ministry of Higher Education Malaysia through the Long-term Research Grant Scheme – Malaysia Research University Network (LRGS-MRUN), under project code 6300218 (UPM vote number). Financial support for Muhamad Fikri Shazlan Saad was provided through this grant. The authors would like to express their gratitude to the staff of the Anatomy and Histology Laboratory for their outstanding technical assistance.

References

Alharbi, H. F., Algonaiman, R., Alduwayghiri, R., Aljutaily, T., Algheshairy, R. M., Almutairi, A. S., ... & Barakat, H. (2022). Exposure to bisphenol A substitutes, bisphenol S and bisphenol F, and its association with developing obesity and diabetes mellitus: A narrative review. *International Journal of Environmental Research and Public Health, 19*(23), 15918. https://doi.org/10.3390/ijerph192315918

Belcher, S. M., Gear, R. B., & Kendig, E. L. (2015). Bisphenol A alters autonomic tone and extracellular matrix structure and induces sex-specific effects on cardiovascular function in male and female CD-1 mice. *Endocrinology, 156*(3), 882-895. https://doi.org/10.1210/en.2014-1847

Bousoumah, R., Leso, V., Iavicoli, I., Huuskonen, P., Viegas, S., Porras, S. P., ... & Ndaw, S. (2021). Biomonitoring of occupational exposure to bisphenol A, bisphenol S and bisphenol F: A systematic review. *Science of the Total Environment, 783,* 146905. https://doi.org/10.1016/j.scitotenv.2021.146905

Cano-Nicolau, J., Vaillant, C., Pellegrini, E., Charlier, T. D., Kah, O., & Coumailleau, P. (2016). Estrogenic effects of several BPA analogs in the developing zebrafish brain. *Frontiers in Neuroscience, 10*, 112. https://doi.org/10.3389/fnins.2016.00112

Chen, J. (2013). Impaired cardiovascular function caused by different stressors elicits a common pathological and transcriptional response in zebrafish embryos. *Zebrafish, 10*(3), 389-400. https://doi.org/10.1089/zeb.2013.0875

Di Lombo, M. S., Cavalie, I., Camilleri, V., Armant, O., Perrot, Y., Cachot, J., & Gagnaire, B. (2023). Tritiated thymidine induces developmental delay, oxidative stress and gene overexpression in developing zebrafish (Danio rerio). *Aquatic Toxicology, 265,* 106766. https://doi.org/10.1016/j.aquatox.2023.106766

Felisbino, K., Kirsten, N., da Silva Milhorini, S., Marçal, I. S., Bernert, K., Schiessl, R., ... & Guiloski, I. C. (2023). Teratogenic effects of the Dicamba Herbicide in Zebrafish (Danio rerio) embryos. *Environmental Pollution, 334*, 122187. https://doi.org/10.1016/j.envpol.2023.122187

García García, M., Picó, Y., & Morales-Suárez-Varela, M. (2024). Effects of bisphenol A on the risk of developing obesity. *Nutrients, 16*(21), 3740. https://doi.org/10.3390/nu16213740

Gautam, M. K., Panda, P. K., Dubey, A., Kumari, M., & Ghosh, N. S. (2024). zebrafish as a fascinating animal model: a robust platform for in vivo screening for biomedical research. *International Journal of Pharmaceutical Investigation, 14*(3), 696–701. https://doi.org/10.5530/ijpi.14.3.80

Gorelick, D. A., Iwanowicz, L. R., Hung, A. L., Blazer, V. S., & Halpern, M. E. (2014). Transgenic zebrafish reveal tissue-specific differences in estrogen signaling in response to environmental water samples. *Environmental Health Perspectives, 122*(4), 356-362. https://doi.org/10.1289/ehp.1307329

Gyimah, E., Xu, H., Dong, X., Qiu, X., Zhang, Z., Bu, Y., & Akoto, O. (2021). Developmental neurotoxicity of low concentrations of bisphenol A and S exposure in zebrafish. *Chemosphere, 262,* 128045. https://doi.org/10.1016/j.chemosphere.2020.128045

Han, Y., Fei, Y., Wang, M., Xue, Y., Chen, H., & Liu, Y. (2021). Study on the Joint Toxicity of BPZ, BPS, BPC and BPF to Zebrafish. *Molecules, 26*(14), 4180. https://doi.org/10.3390/molecules26144180

Huang, Q., Chen, Y., Lin, L., Liu, Y., Chi, Y., Lin, Y., ... & Dong, S. (2017). Different effects of bisphenol a and its halogenated derivatives on the reproduction and development of Oryzias melastigma under environmentally relevant doses. *Science of the Total Environment, 595*, 752-758. https://doi.org/10.1016/j.scitotenv.2017.03.263

Huang, Q., Fang, C., Chen, Y., Wu, X., Ye, T., Lin, Y., & Dong, S. (2012). Embryonic exposure to low concentration of bisphenol A affects the development of Oryzias melastigma larvae. *Environmental Science and Pollution Research, 19*(7), 2506-2514. https://doi.org/10.1007/s11356-012-1034-6

Huang, W., Wang, X., Zheng, S., Wu, R., Liu, C., & Wu, K. (2021). Effect of bisphenol A on craniofacial cartilage development in zebrafish (Danio rerio) embryos: A morphological study. *Ecotoxicol Environ Saf, 212*(111991), 10-1016. https://doi.org/10.1016/j.ecoenv.2021.111991

Huang, W., Wu, T., & Wu, K. (2022). Zebrafish (Danio rerio): A potential model to assess developmental toxicity of ketamine. *Chemosphere, 291,* 133033. https://doi.org/10.1016/j.chemosphere.2021.133033

Kang, J. H., Asai, D., & Toita, R. (2023). Bisphenol A (BPA) and cardiovascular or cardiometabolic diseases. *Journal of Xenobiotics, 13*(4), 775-810. https://doi.org/10.3390/jox13040049

Karunarathne, W. A. H. M., Molagoda, I. M. N., Choi, Y. H., Park, S. R., Lee, S., & Kim, G. Y. (2021). Bisphenol A: A potential Toll-like receptor 4/myeloid differentiation factor 2 complex agonist. *Environmental Pollution, 278*, 116829. https://doi.org/10.1016/j.envpol.2021.116829

Khalili Sadrabad, E., Hashemi, S. A., Nadjarzadeh, A., Askari, E., Akrami Mohajeri, F., & Ramroudi, F. (2023). Bisphenol A release from food and beverage containers–A review. *Food Science & Nutrition, 11*(7), 3718-3728. https://doi.org/10.1002/fsn3.3398

Lombó, M., Fernández-Díez, C., González-Rojo, S., Navarro, C., Robles, V., & Herráez, M. P. (2015). Transgenerational inheritance of heart disorders caused by paternal bisphenol A exposure. *Environmental Pollution, 206,* 667-678. https://doi.org/10.1016/j.envpol.2015.08.016

Maćczak, A., Cyrkler, M., Bukowska, B., & Michałowicz, J. (2017). Bisphenol A, bisphenol S, bisphenol F and bisphenol AF induce different oxidative stress and damage in human red blood cells (in vitro study). *Toxicology in Vitro, 41*, 143-149. https://doi.org/10.1016/j.tiv.2017.02.018

Mu, X., Huang, Y., Li, X., Lei, Y., Teng, M., Li, X., ... & Li, Y. (2018). Developmental effects and estrogenicity of bisphenol A alternatives in a zebrafish embryo model. *Environmental Science & Technology, 52*(5), 3222-3231. https://doi.org/10.1021/acs.est.7b06255

Oliviero, F., Marmugi, A., Viguié, C., Gayrard, V., Picard-Hagen, N., & Mselli-Lakhal, L. (2022). Are BPA substitutes as obesogenic as BPA? *International Journal of Molecular Sciences, 23*(8), 4238. https://doi.org/10.3390/ijms23084238

Park, C. G., Adnan, K. M., Cho, H., Ryu, C. S., Yoon, J., & Kim, Y. J. (2024). A combined in vitro-in silico method for assessing the androgenic activities of bisphenol A and its analogues. *Toxicology in Vitro, 98,* 105838. https://doi.org/10.1016/j.tiv.2024.105838

Perumal, V., Khatib, A., Ahmed, Q. U., Uzir, B. F., Murugesu, S., Primaharinastiti, R., ... & Selamat, J. (2022). Toxicity and teratogenicity evaluation of ethanolic extract from Momordica charantia fruit using zebrafish (Danio rerio) embryo model. *International Food Research Journal, 29*(3), 531-539. http://dx.doi.org/10.47836/ifrj.29.3.06

Pinto, C., Grimaldi, M., Boulahtouf, A., Pakdel, F., Brion, F., Aït-Aïssa, S., ... & Balaguer, P. (2014). Selectivity of natural, synthetic and environmental estrogens for zebrafish estrogen receptors. *Toxicology and Applied Pharmacology, 280*(1), 60-69. https://doi.org/10.1016/j.taap.2014.07.020

Pinto, C., Hao, R., Grimaldi, M., Thrikawala, S., Boulahtouf, A., Ait-Aissa, S., ... & Bondesson, M. (2019). Differential activity of BPA, BPAF and BPC on zebrafish estrogen receptors in vitro and in vivo. *Toxicology and Applied Pharmacology, 380,* 114709. https://doi.org/10.1016/j.taap.2019.114709

Qiu, W., Zhan, H., Hu, J., Zhang, T., Xu, H., Wong, M., ... & Zheng, C. (2019). The occurrence, potential toxicity, and toxicity mechanism of bisphenol S, a substitute of bisphenol A: A critical review of recent progress. *Ecotoxicology and Environmental Safety, 173*, 192-202. https://doi.org/10.1016/j.ecoenv.2019.01.114

Rosenmai, A. K., Dybdahl, M., Pedersen, M., Alice van Vugt-Lussenburg, B. M., Wedebye, E. B., Taxvig, C., & Vinggaard, A. M. (2014). Are structural analogues to bisphenol a safe alternatives? *Toxicological Sciences, 139*(1), 35-47. https://doi.org/10.1093/toxsci/kfu030

Thiagarajan, S. K., Rama Krishnan, K., Ei, T., Husna Shafie, N., Arapoc, D. J., & Bahari, H. (2019). Evaluation of the effect of aqueous Momordica charantia Linn. extract on zebrafish embryo model through acute toxicity assay assessment. *Evidence-Based Complementary and Alternative Medicine, 2019*(1), 9152757. https://doi.org/10.1155/2019/9152757

Wu, L. H., Zhang, X. M., Wang, F., Gao, C. J., Chen, D., Palumbo, J. R., ... & Zeng, E. Y. (2018). Occurrence of bisphenol S in the environment and implications for human exposure: A short review. *Science of the Total Environment, 615*, 87-98. https://doi.org/10.1016/j.scitotenv.2017.09.194

Yang, X., Yan, Z., Chen, J., Wang, D., & Li, K. (2023). Acute toxicity of the dinoflagellate Amphidinium carterae on early life stages of zebrafish (Danio rerio). *Toxics, 11*(4), 370. https://doi.org/10.3390/toxics11040370

Yin, N., Liang, X., Liang, S., Liang, S., Yang, R., Hu, B., ... & Faiola, F. (2019). Embryonic stem cell-and transcriptomics-based in vitro analyses reveal that bisphenols A, F and S have similar and very complex potential developmental toxicities. *Ecotoxicology and Environmental Safety, 176,* 330-338. https://doi.org/10.1016/j.ecoenv.2019.03.115

Yuan, M., Chen, S., Zeng, C., Fan, Y., Ge, W., & Chen, W. (2023). Estrogenic and non-estrogenic effects of bisphenol A and its action mechanism in the zebrafish model: An overview of the past two decades of work. *Environment International, 176,* 107976. https://doi.org/10.1016/j.envint.2023.107976