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The effect of bisphenol A and its analogue, bisphenol S on stress response of developing zebrafish (*Danio rerio*)

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Bisphenol A (BPA) is a widely used industrial chemical and a xenoestrogen that poses significant biological effects in living organisms. Owing to the health effects of BPA exposure, Bisphenol S (BPS) was introduced as a safe alternative. However recent research has acclaimed the endocrine disruptive ability and negative health effects of BPS, raising concerns on the safety of BPS. Therefore, comparative assessments on the biological effect of BPA and BPS are essential. This study was conducted to comparatively assess the effects of bisphenols on the physiological stress of model organism, zebrafish. The stress response refers to a coordinated series of physiological and behavioural reactions in an animal that helps to restore internal homeostasis disturbed by environmental stressors. Hence behavioural and physiological assays are used in determining the stress level of zebrafish. Swimming activity and aggression are important behaviours influenced by physiological stress. Ammonia is an end product of protein metabolism, which is considered a primary universal waste product. Ammonia excretion is an essential physiological parameter to assess the physiological stress of aquatic organisms. The research was conducted to investigate the comparative impact of BPA and BPS on stress response by examining the swimming performance, aggression (mirror-biting test) and ammonia excretion of juvenile zebrafish. The study was conducted with 21-days old zebrafish, exposed to environmentally relevant concentrations of BPA (50 µg/L), BPS (50 µg/L) and treatment control for 63 days. The swimming speed, aggression and ammonia excretion were determined at the end of the exposure period. According to the results, the mean maximum swimming speeds of fish exposed to BPA (0.40 m/s) and BPS (0.36 m/s) were significantly lower than that of the control (0.56 m/s, $p<0.05$) and a notable difference was not observed between BPA and BPS exposed fish. The mirror-biting test indicated that both bisphenols showed higher aggression than the control (1.2 bitings/minute, $p < 0.05$) while BPA (98.8 bitings/minute) showed significantly higher aggression than BPS (48.3 bitings/minute, $p<0.05$). The ammonia excretion of BPA-exposed fish (1.161 ppm) and BPS-exposed fish (1.055 ppm) was considerably higher than the control (0.384 ppm, $p<0.05$), and a significant difference was not observed between BPA and BPS-exposed fish ($p>0.05$). In conclusion, both BPA and BPS can similarly reduce swimming speed and increase ammonia excretion as a response to stress induced by these bisphenols. However, the mirror-biting test, which measures the aggression level of fish implies that even if both bisphenols produce significant aggression, BPA causes notably higher aggression levels than BPS. The findings of the study suggest that BPS cannot be recommended as a safe alternative to BPA as both bisphenols potentially induce physiological and behavioural stress. More comprehensive physiological and cellular assays are encouraged to further comprehend the comparable effects of BPA and BPS on the physiological stress of aquatic organisms.

Keywords: Ammonia, BPA, BPS, Stress response, Zebrafish (*Danio rerio*)

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