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Assessment of Toxic Effects of the Endocrine Disruptor Bisphenol-A on Zebra Fish (Danio Rerio) - A Vertebrate System

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Abstract: ***Bisphenol A (BPA) is considered to be one of the important component of plastics, BPA is regarded to be an endocrine disruptor thereby interfering with the production, secretion, transport, action, function and elimination of natural hormones. In the present study the toxic effects of BPA was assessed on mature adult zebra fishes and young fragile embryos. Zebra fishes were grown in reverse osmosis water containing known concentrations of BPA ranging from 0.01Molar (M) to 0.1M. After spawning the young fragile embryos were also subjected to BPA treatment the result of which was mortality of the adult fishes and young embryos. The toxicity of the same was determined in-vivo by measuring the acetyl cholinesterase (AChE) activity in the brain tissue of zebra fish. The results projected a very high AChE activity indicating neuro transmission obstruction and mortality of the zebra fishes.***

Keywords: BPA, Acetyl cholinesterase (AChE), Zebra fish, plastic toxicology

1. Introduction

Globalization and commercialization has enrooted luxurious development amidst society. This has led to large scale use of plastics and in turn BPA. The use of plastic in packaging industry and household goods has stabilized the BPA content on planet earth (Raj et al. 2015). BPA is a part of many materials like plastic containers; carry bags, water bottles etc. Bisphenol A is an important chemical used to harden plastic. Polycarbonates are plastics which are very hard, so hard they are used to make screwdriver handles, shatter-proof eyeglass lenses, and other very durable products. Hard plastics remain unchanged in the environment for decades or centuries. Biodegradation, of course, releases BPA to the environment (saido et al.2010). Many studies of consumer products identified sources of human exposures including plastics, dental sealants, food contact papers and canned foods (Vandenberg et al., 2008). The highest amounts of BPA is reported in landfill leachate and pulp mill effluents up to 17 mg/L; (Flint et al. 2012), and in surface waters low concentrations of BPA are found (Crain et al. 2007; Flint et al. 2012). BPA has been detected in river and marine sediments (Flint et al. 2012 and Koh et al. 2006). This affects the aquatic biota in changing its morphology, behavior and accumulation of BPA in the blood this in turn affects human being when these fishes are consumed. Bioremediation helps in cleaning up the natural ecosystem and maintains a homeostatic balance; it was observed that Pistia stratiotes has the ability to phytoremediate BPA from water (raj et al. 2015). BPA levels if left unmonitored will make its entry into the food chain of human beings and other life forms and it behaves as an endocrine disruptor, causing chromosome damage and also influences the biochemical mechanisms pertaining obesity (Takeuchi et al 2004). It also affects plants and animals which makes ecological imbalance in the environment by changing its behavior and morphology. Focusing on the wide usage of Bisphenol A the objective of the study was to evaluate the toxic effects of BPA on zebra fish a vertebrate system.

2. Materials and Methods

Preparation of BPA Solution

Bisphenol A is a colorless solid which is poorly soluble in water hence 1M sodium hydroxide (NaOH) was used to dissolve. NaOH (1M) was added drop wise until BPA dissolved and the homogenous solution was made up to 100ml. Known concentrations of 0.01M, 0.02M, 0.04M, 0.06M, 0.08M and 0.1M of BPA were prepared.

Zebra fish analysis

Breeding variety of zebra fishes were procured from aquarium shops and grown in small tanks. The tanks were well aerated and also fitted with a sieve below to trap the eggs after early morning spawning. Eggs were taken into small glass petriplates containing reverse osmosis water and the water samples containing various known concentrations of BPA to determine heartbeat count, morphological changes and to assess AChE activity

Assay for acetylcholine esterase activity

Male adult fishes were grown in reverse osmosis water which was kept as control water sample and then in the water samples with different known concentrations of BPA. These fishes were euthanized to dissect the brain cells and the brain cells were homogenized at 4° C with phosphate buffer. The homogenate was subjected to centrifugation and the supernatant was taken as the enzyme source. 20X dilution of the supernatant were prepared and then the Enzyme activity was estimated by titration method (Guruprasad et al., 2014). Acetylcholine iodide and sodium hydroxide were used as the reagents with phenolphthalein as the indicator.

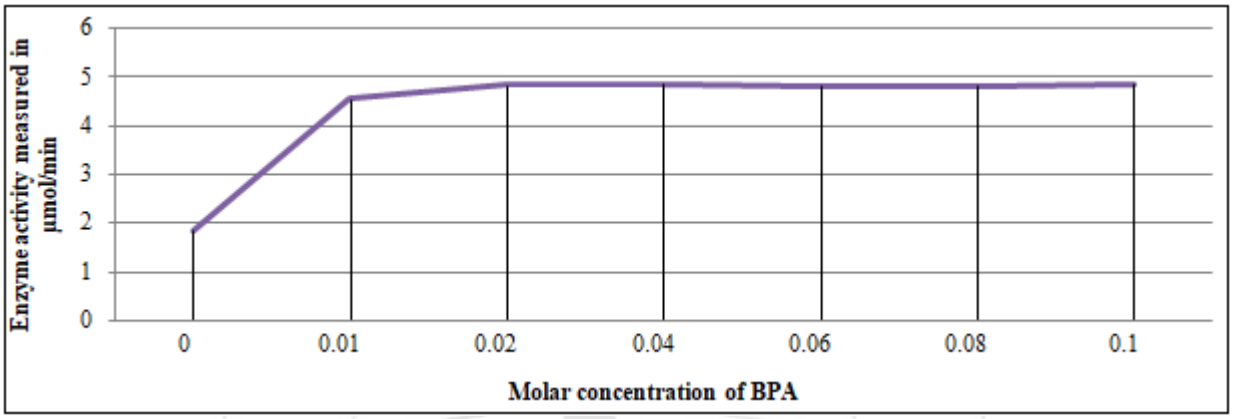
3. Results and Discussion

Table 1: indicating the heart beat count recorded in zebra fish embryos exposed to various known concentrations of BPA

Sample	Concentration of BPA	Heart beat recorded per minute
Control	0.0M	146/minute
sample-1	0.01M	-
sample-2	0.02M	-
sample-3	0.04M	-
sample-4	0.06M	-
sample-5	0.08M	-
sample-6	0.1M	-

Table 2: indicating the AChE activity from brain cells of zebra fish treated with various concentrations of BPA

Sample	*Concentration of BPA*	*Enzyme activity (µmol/min)*
Control	0.0M	1.834 µmol/min
sample-1	0.01M	4.543 µmol/min
sample-2	0.02M	4.824 µmol/min
sample-3	0.04M	4.843 µmol/min
sample-4	0.06M	4.813 µmol/min
sample-5	0.08M	4.813 µmol/min
sample-6	0.1M	4.843 µmol/min



Graph 1: indicating the AChE activity from brain cells of zebra fish treated with various concentrations of BPA

The morphological changes and the heartbeat count was unable to be recorded, the young fragile embryos immediately died when exposed to the water samples containing different concentrations of BPA. 145heartbeats per minute was recorded in the control water sample. The acetylcholine esterase activity was found to be very high which is an indication that acetylcholine was degraded at a faster rate. These results indicate that neurotransmission is hampered because of alkaline water samples with BPA as one of the pollutant. Stress in neurotransmission leads to loss of metabolism, tremors, changes in the body weight, stiffness and many other diseases.

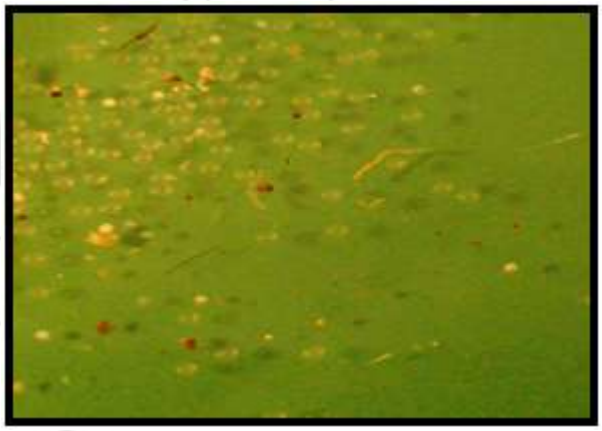


Figure 1: Showing zebra fish embryos

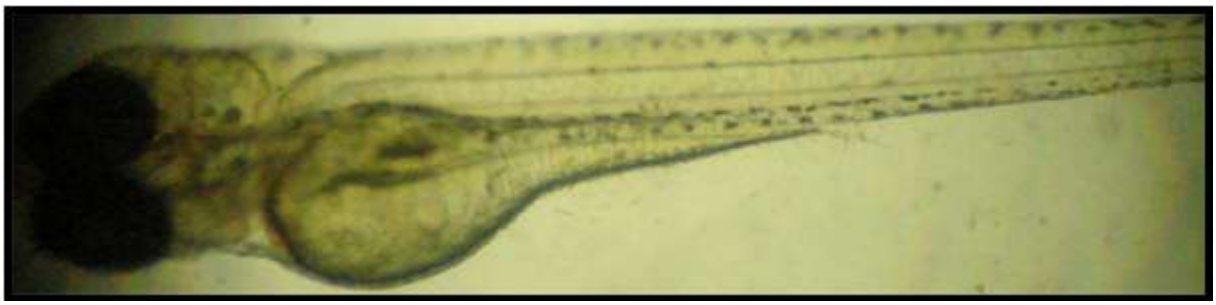


Figure 2: Showing fully developed embryo observed at 10x magnification in control water sample

Figure 3: Showing zebra fish in control set up

Figure 4: Dead zebra fish after exposure to alkaline water containing BPA

4. Conclusion

The present investigation reports alkaline waters can easily dissolve BPA and other components of plastic. Acetylcholine esterase activity from different fishes exposed to different concentrations of BPA showed a very high enzyme activity suggesting BPA to be neurotransmission disruptor and an endocrine disruptor. Highlighting the toxicity of BPA on a vertebrate model like zebra fish is an indication that BPA can cause harmful effects such as hampering neuro transmission, morphological change and reduced heart beat resulting in death. The same effects would be observed in higher life forms including human beings when exposed to BPA. Experimental studies showcase the effects of alkaline water and BPA. Reduction, recycling and reusing plastics would bring down the use of Bisphenol A. Awareness amidst the society should be created about effects of BPA and Preventive methods should be employed to cut down the use of plastics and in turn BPA, Natural methods like phytoremediation and microbial remediation should be implemented to remediate and reduce BPA from water and soil.

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Mathews P Raj did MSc. Botany, Mphil. Biotechnology. Presently he is Assistant Professor- Department of Microbiology and Botany, School of Sciences, Jain University. Published 7 Research articles in international journals and have filed 1Patent. He has 11 Funded projects (Jain RDC, St. Joseph's college, VGST, Green peace India, DFTR)