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Acute Toxicity Assessment of Sodium Arsenite in Zebrafish (*Danio rerio*) Under Ideal Physico-Chemical Conditions

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Abstract:- Arsenic is ubiquitously prevalent metalloid contaminant released in the aquatic environment as a consequence of geogenic and anthropogenic activities. After West Bengal, Bihar has emerged as a new hotspot for arsenic toxicity in India. Presence of arsenic in groundwater and soil in various forms has been a matter of concern for the scientific community globally. The present study aims to assess the median lethal concentration (LC_{50}) for sodium arsenite ($NaAsO_2$) in *Danio rerio* (zebrafish). Healthy fish were procured from Ashish Aquarium Traders, Howrah, India, acclimated in the laboratory for 15 days in ideal laboratory condition and fed *ad libitum*. Following a range finding test for arsenic, a set of doses were administered to the fish for 24h, 48h, 72h and 96h. The LC_{50} for each assessment period was separately determined using probit regression analysis and later confirmed by pilot test. The24h, 48h, 72h and 96h LC_{50}were determined as 45mg/L, 44mg/L, 41mg/L and 39mg/L respectively. Since there is great similarity in genome of zebrafish and humans, the finding of the present acute toxicity study may be interpreted for the humans.

***Keywords**:- Acute Toxicity, Anthropogenic, Danio Rerio, Geogenic, LC_{50}, Physic-Chemical, Probit Regression, Sodium Arsenite.*

I. INTRODUCTION

Aquatic ecosystem has been subjected to immense anthropogenic load in terms of non-judicious applications of various synthetic agrochemicals including heavy metals[1][2][3][4]. Heavy metals are those that have a relatively high density compared to water (>5g/cc^3) and their atomic weight is 63.5-200.5 g/mol[5]. Heavy metal contamination of the environment results from both natural sources and human activities. They are not completely metabolised by the living system and as such they accumulate in body tissues and disturb normal physiology. In fishes they take their entry via general body surface, gills or digestive tract and induces various physiological, biochemical and oxidative perturbations[1][6][7].

Weathering of rock, leaching, run off, volcanic and biological activities include the natural resources of fresh water contamination[8][9]. Major anthropogenic sources of groundwater arsenic contamination are extensive mining and geothermal activities, fossil fuel combustion, use of metallic arsenite for strengthening alloys as well as in the processing of glass pigments, textiles, metal adhesive wood preservatives, ammunition and other synthetic arsenical compounds[10][11]. More than 230 million people worldwide are suffering due to arsenic contaminated consumption of water including 180 million people from Asia alone. In India, around 50 million people living in 20 states have been reported to be affected by arsenic contaminated groundwater consumption[12]. The soil and groundwater concentration of arsenic in some of these states adjacent to Ganga-Brahmaputra basin have been found to be far above the limit specified by WHO *i.e*.,10ppb[13][14][15]. It is known to cause gall bladder, urinary bladder, skin and liver cancer and various other physiological abnormalities. It is known to disrupt physiological processes via induction of oxidative stress. It creates imbalance between RONS (reactive oxygen and nitrogen species) generation and annihilation. Arsenic induced oxidative stress is reported to damage cell membrane and DNA while also affecting major cell organelles including Golgi complex and ER[7][16][17][18].

In nature, arsenic is present as both arsenite (3+) and arsenate (5+). Arsenite is reported to be comparatively more toxic form[12]. Since fishes are best sentinels to study the pollution status of an aquatic body, they have been widely used to assess water quality of aquatic ecosystem[19][20]. Following heavy metal toxicity, the growth rate, reproductive efficacy and other metabolic functions of the fish are hindered, ultimately causing mortality in fishes[21].

Zebrafish is a tropical freshwater fish in the minnow family. Since past few decades, it has become a preferred model organism for human disease research as 70% of the protein-coding human genes are related to genes found in zebrafish[22]. Several research has revealed that zebrafish undergo similar changes in its enzymatic profile as humans when exposed to heavy metals[23][16][24]. The present study has been carried out to assess the acute toxicity of sodium arsenite in zebrafish in ideal laboratory conditions. It is also focussed on the study of behavioural changes and corresponding stress responses of the fish during acute toxicity test.

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II. MATERIAL AND METHOD

A. Experimental animal:

Danio rerio with an average weight of 0.409 ± 0.02g and length 3.5 ± 0.05 cm were purchased from Ashish Aquarium Traders, Howrah, India, in June 2022. Fish were given a bath of 0.1% $KMnO_4$ solution, segregated as per size in 20L aquaria and acclimated for 15 days in the laboratory. They were fed daily *ad libitum.*

B. Experimental Design:

A total of 240 fish were used in the experiment. The fish were divided into 8 groups of 10 fish each in 20L aquarium capacity- 7 experimental groups and 1 control group. The test was performed in triplicate.

The physico-chemistry of water *i.e.*, temperature, pH, dissolved oxygen were analysed as per the standard protocol of APHA (2017).

C. Preparation of stock solution and dose administration:

Sodium arsenite ($NaAsO_2$, 98.9% pure) was purchased from Loba Chemie Pvt. Ltd. Stock solution of 20,000 mg/L was prepared by dissolving $NaAsO_2$ in distilled water. The test solution used in 7 different groups ranged from 20mg/l to 50mg/l. The feeding of the fish was stopped during the test. The test solution was renewed every day in order to maintain the desired concentration in each group. The fish were examined closely for any behavioural or morphological changes. The dead fish were removed immediately to prevent the contamination of water.

D. Statistical Analysis:

The percentage mortality at 24, 48, 72 and 96 hours were recorded. The corresponding LC_{50} values and its confidence limits (95% CL_S) were calculated by Finney's probit regression method and confirmed by pilot test. To determine the relationship between logarithm of concentration and the corresponding percentage mortalities, the regression equation was obtained using MS Excel Office 2021 software. The antilog of concentration gave the value of LC_{50}. All tests were performed in triplicate.

III. RESULTS AND DISCUSSION

To study the acute toxicity of sodium arsenite in *Danio rerio*, doses of 20, 25, 30, 35, 40, 45 and 50 mg/L of the test chemical were administered to the fish of different aquaria. The percentage mortalities were recorded at the end of 24 h, 48 h, 72 h and 96 h (table1 and figure 1).

Table 1. Percentage death of fish exposed to different concentrations of sodium arsenite for different durations.

Conc. (mg/L)	Log conc.	% mortality in 24 h	Probit kill	% mortality In 48 h	Probit kill	% mortality In 72 h	Probit kill	% mortality In 96 h	Probit kill
20	1.30103	0	0.00	0	0.00	0	0.00	0	0.00
25	1.39794	10	3.72	0	3.72	0	3.72	0	3.72
30	1.47712	20	4.16	0	4.16	0	4.16	10	4.48
35	1.54406	20	4.16	0	4.16	10	4.48	10	4.75
40	1.60206	30	4.48	0	4.48	10	4.75	10	5.00
45	1.65321	20	4.16	0	4.16	20	4.75	10	5.00
50	1.69897	60	5.25	10	5.52	10	5.84	0	5.84

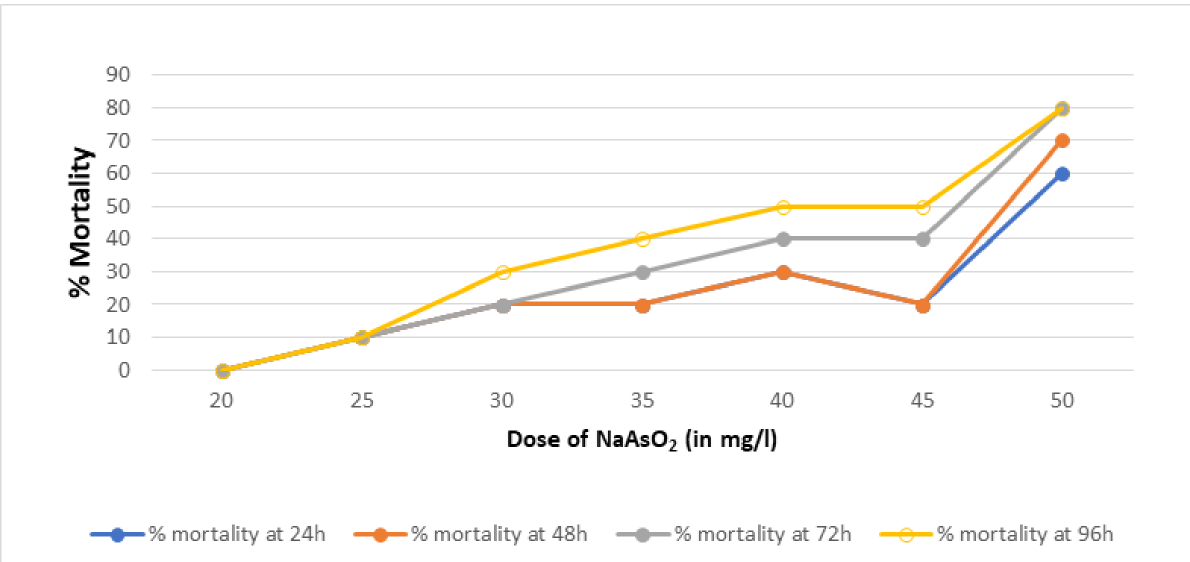
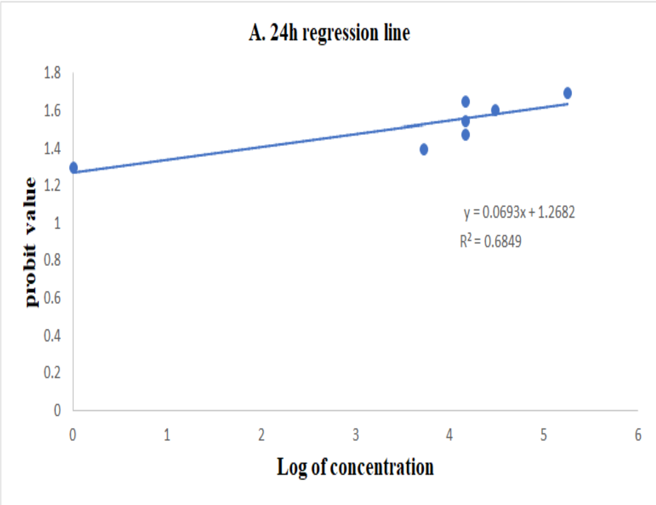


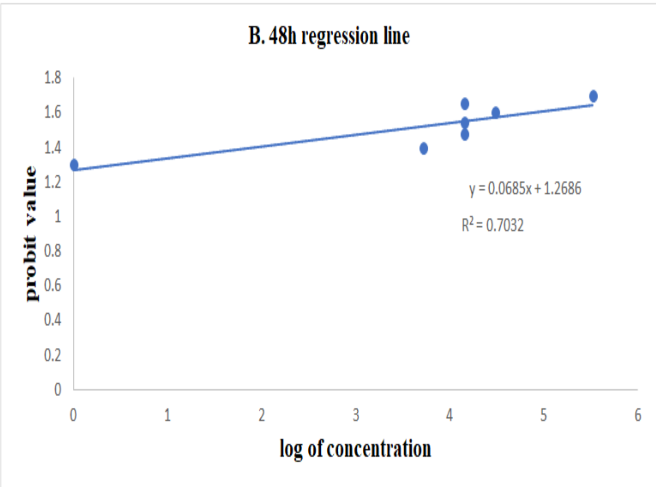
Fig. 1 Mortality (%) of fish exposed to different concentrations of $NaAsO_2$

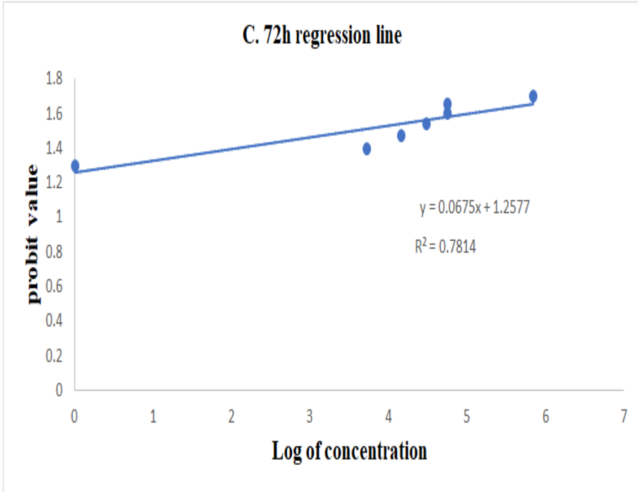
Table 2. The corresponding 24h, 48h, 72h and 96h LC_{50} values for $NaAsO_2$ in *Danio rerio*.

S.No	Exposure period	LC_{50} (mg/L)
1.	24 hours	45.289
2.	48 hours	44.400
3.	72 hours	41.209
4.	96 hours	39.719

It is evident that the mortality is positively correlated with dose. There was no mortality in the control group. There is marked difference in LC_{50} values at each successive 24 hour. The percentage mortality was converted into probit units and plotted against the logarithm of concentration of sodium arsenite to obtain a regression line and regression equation for each time interval (figure 2A, 2B, 2C and 2D).







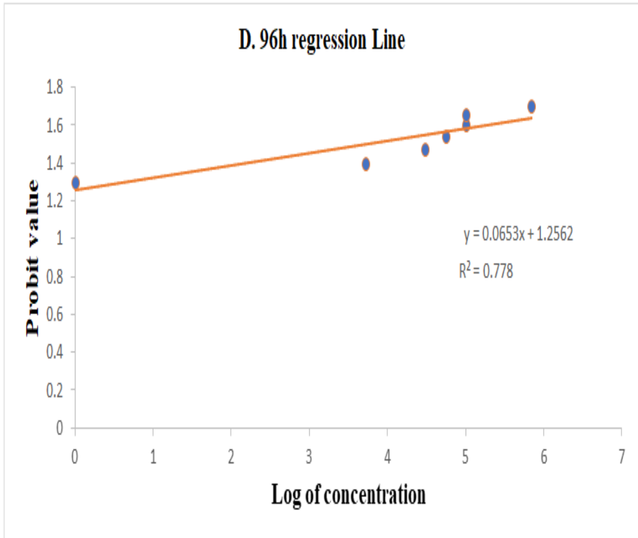


Fig 2. Regression line between probit kill and log concentration for different time intervals [A-24h, B-48h, C-72h, D-96h] in $NaAsO_2$ treated zebrafish.

Zebrafish, belonging to Cyprinidae family, is a popular freshwater fish native to South Asia. In recent years it has become one of the most preferred model organisms due to various reasons[24]. The present study revealed the 24h, 48h, 72h and 96h LC_{50} value of sodium arsenite to the fish as 45mg/L, 44mg/L, 41mg/L and 39mg/L respectively (table 2).

The findings of the present investigation show dose- and duration-dependent mortality in zebrafish. At 48 hours there is slight decline in mortality from 30% to 20% as the dose increases from 40mg/L to 45mg/L, suggesting an increase in oxidative stress resistance mechanism in fish after a certain threshold dose. The anti-oxidants might have attained a peak following 48-hour exposure to 40mg/L of sodium arsenite, which was the probable cause for decline in mortality till an exposure to 45mg/L. The fish failed to counter oxidative stress generated by further higher concentration of the toxicant exposure and therefore there has been no decline in mortality following 48 hours.

The acceptable limit of total arsenic concentration is 340 µg/L for acute exposure and 150 µg/L for chronic exposure in freshwater as per USEPA aquatic life criteria. However, according to NAS (1977), a concentration of 1.5-3.8 mg/L of arsenite has been considered safe for the fish. The concentration of 13.6 mg/L of As (III), used for effective weed control has been considered to be harmful to several fish species[25][26].

Acute toxicity is the discernible adverse effect induced by a substance in an organism within a short time of exposure[21][27]. It is the effect that can damage organs due to exposure of chemical/ toxicant in a short time[28].

IV. CONCLUSION

The findings of the present study provide an insight of acute toxicity range of sodium arsenite to the fresh water fish *Danio rerio*. The LC_{50} value determined in the present acute toxicity assessment is much higher than their environmental relevance, still it contributes a lot in determination of dose and duration of AS(III) to the freshwater fish while studying the molecular mechanism related to oxidative and genotoxic potentials of the toxicant to the fish. Acute toxicity test is highly appreciated to predict the safe concentration of a particular toxicant in the environment.

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➢ *Conflict of Interest*

The authors declare no conflict of interest.

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