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Short Communication

Effects of arsenic trioxide on *Poecilia sphenops*

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Abstract

Keratosis, skin cancer and neurological disorder in human and experimental animals are among the most commonly observed health effects associated with acute and chronic exposures to inorganic arsenic. This study examines the acute and chronic dose-responses of arsenic trioxide on Poecilia sphenops (Black Molly). P. sphenops has been reported as a highly adaptable species and robust in nature. It has been extensively used to evaluate toxicity of many pollutants due to their high growth rate, birth size, reproduction and brood number. In current investigations, a batch experiment was conducted under control conditions to assess the effect of As_2O_3 on Black Molly. Lethal concentration (LC_{50}) of As_2O_3 were investigated and observed to be 49.5 mg/L. Growth performance, macroscopic observation and mortality of fingerlings of P. sphenops in experimental tank containing 1 mg/L of As_2O_3 was monitored for 60 days. Mortality of fishes in the experimental tank was observed to be ~20% after 60 days of incubations. Significant change in morphometric, behavior and diminution in growth performance were observed. These results evidently indicate the sensitivity of Black Molly towards As_2O_3.

Keywords: Arsenic Trioxide, Black Molly, LC_{50}, Morphometric and *Poecilia sphenops.*

Introduction

Arsenic is a metalloid chemical element and natural component of the earth's crust which occurs in many minerals, usually in conjunction with sulfur and other metals, and also as a pure elemental crystal. It has been observed to be widely distributed throughout the environmental compartments including air, water and soil[1]. This is possibly due to weathering of rocks, leaching, runoff and anthropogenic activities. The main industrial uses of metallic arsenic are for strengthening alloys as well as in the processing of glass, pigments, textiles, paper, metal adhesive, wood preservatives and ammunition[2]. In general, inorganic arsenic is more toxic than organic form of arsenic to aquatic animals and trivalent species are reported to be more toxic than pentavalent species[3]. It isused in the production of pesticides, treated wood products, feed additives, herbicides, insecticides and pharmaceuticals[4]. Human beings are exposed to elevated levels of inorganic arsenic through water, foodand smoking tobacco. As_2O_3 has been reported in varying concentrations in almost all the environmental compartment possibly due to extensive use and dispose of arsenic containing products.

In current investigations, effect of As_2O_3 on growth and development of *Poecilia sphenops* was conducted in batch mode under control conditions. *P. sphenops* commonly known as "Black Molly" is an omnivorous ornamental fish. It has been reported to be highly adaptable and extensively thrive in many different environmental and ecological conditions as a model organism for the studies of ecology, evolution and behavioral due to their viviparous in nature and easy availability[5,6]. Being a tolerant species these mollies are able to exploit the thin film of oxygen rich surface water with their mouths, and can survive in low oxygen environment.

Materials and methods

Source of chemicals: More than 99% pure analytical grade As_2O_3 was purchased from Merck, India. All other chemicals were of high purity (>99%). Commercial pellet feed were purchased from Aini Pellet feed for ornamental fishes, China. All experiments were performed in duplicate unless otherwise specified.

Source of fish and thriving conditions: Mixed population of *P. sphenops* was procured and transported in 5 L of plastic bags filled with 3 L of water from Sagar Aquarium, Vadodara, India to a holding facility at the Environmental Monitoring Laboratory, Department of Environmental Studies, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, India. The procured fishes were further cultivated in 20 L fish culturing experimental tank containing 10 L of water under control conditions with commercial pellet feed for further studies. The experimental tanks were located in an outdoor area, covered with a lid to prevent the entry of dust and rain water, and the fish were fed once a day. Dose of pellet feed was 10 mg/fish/day. Aeration was provided to all the experimental tanks with the help of aerators. During the experiment, the water quality parameters were analyzed periodically to monitor the health of the fishes. The mean values for temperature, dissolved oxygen, pH, and hardness were 21 ± 4°C, 4.85 ± 0.5 mg/L, 7.8 ± 0.2 and 248 ± 5 mg/L, respectively.

Characterization of fish: Morphometric characteristics of fish such as length, width and height of fishes were calculated with the help of thread, scale and Vernier Calliper measurement method. Figure-1 shows standard length, body width, caudal penduncle length, caudal penduncle depth, head length, head width, head depth, snout length (pre-orbital distance), eye diameter, postorbital distance, inter-orbital distance, pre-dorsal distance, post-dorsal distance, pre-pelvic distance, post-pelvic distance, pre-anal distance, post-anal distance, dorsal fin base, dorsal fin length, anal fin length, anal fin base, pectoral fin length, pelvic fin length, pectoral ventral distance, mouth width and pre-maxilla length of molly fish.

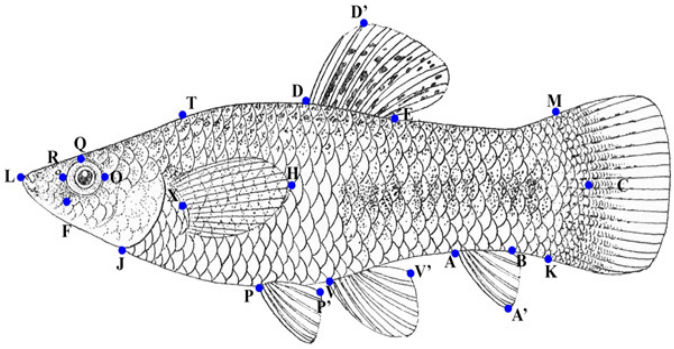

Figure- 1: Schematic diagram of molly fish showing morphometric characteristics LC: Standard length, D↓: Body width, BC: Caudal penduncle length, B↑: Caudal penduncle depth, LH: Head length, XX': Head width, TJ: Head depth, LR: Snout length (pre-orbital distance), RO: Eye diameter, OH: Postorbital distance, QQ': Inter-orbital distance, DL: Pre-dorsal distance, EM: Post-dorsal distance, LV: Pre-pelvic distance, VK: Post-pelvic distance, LA: Pre-anal distance, BK: Post-anal distance, DE: Dorsal fin base, DD': Dorsal fin length, AA': Anal fin length, AB: Anal fin base, PP': Pectoral fin length, VV': Pelvic fin length, PV: Pectoral ventral distance, FF': Mouth width, LF: Pre-maxilla length.

At the end of each experiment, morphological characteristics, survival rate and specific growth rate of the fish were measured and subjected to statistical analysis. Survival rate, mortality rate and specific growth rate was calculated according to equation 1, 2 and 3, respectively.

$$S = \frac{N_f}{N_i} \times 100 \quad (1)$$

In equation 1, S is the survival rate in percentage, N_f is the final number of fishes and N_i is the initial number of fishes.

$$M = \frac{N_i - N_f}{N_i} \times 100 \quad (2)$$

In equation 2, M is the mortality rate in percentage, N_i is the initial number of fishes and N_f is the final number of fishes.

$$\mu = \frac{W_f - W_i}{W_i \times t} \times 100 \quad (3)$$

In equation 3, μ is the specific growth rate in percentage per day, W_f is the final body weight of fish in mg, W_i is the initial body weight of fish in mg and t is the total days of incubation under experimental conditions.

Experimental design and conditions: Our experimental design consisted of a total of forty fingerlings of *P. sphenops* (averaging 92 ± 7 mg in weight and 11.5 ± 4 mm in length), divided into two groups of twenty. One group was exposed to the experimental water containing 1 mg/L of As_2O_3, and second group was exposed to experimental water without arsenic, as control. As_2O_3 was first dissolved in half liters of water taken from the experimental tank, and then this concentrated As_2O_3 solution was mixed with the remaining water (9.5 L) in the tanks. The fish were fed with commercial pellet feed once a day. Dose of pellet feed was 10 mg/fish/day. Aeration was provided to all the experimental tanks with the help of aerators. The tank was kept in such a manner that maximized natural conditions in the laboratory. The experimental tanks were maintained under control conditions for 60 days to observe change in behavior and death of the fishes. In order to study anatomical and morphometric characteristics, fishes were sacrifice after 60 days of incubations.

Determination of lethal concentration of As_2O_3: Toxicity tests were performed in accordance with the standard methods given in APHA[7]. These batch experiments were conducted in 20 L fish tank containing 10 L of water to assess the lethal concentration (LC_{50}) values of As_2O_3 on *P. sphenops*. The fish culturing conditions and tank maintenance were same as discussed in section *B* except the feed was omitted. The initial concentrations of As_2O_3 used were 25, 50, 100 and 200 mg/L. One additional experimental tank was maintained without any concentrations of As_2O_3 as control. Aeration was provided to all the tanks with the help of aerators. Ten juvenile fishes of *P. sphenops* in each experimental tank were introduced. Behavioral changes and mortalities were recorded at 24, 48, 72 and 96 hours of exposure, and dead fish were removed immediately from the experimental tank. LC_{50} was calculated by Probit analysis Finney method[8] on the basis of mortality of *P. sphenops* in each experimental tank.

Results and discussion

Effect of As_2O_3 on P. sphenops under normal feeding condition: Growth performance and macroscopic observation of fingerlings of *P. sphenops* in experimental tank containing As_2O_3 and fed with commercial pellet feed was monitored for 60 days. The fingerlings of *P. sphenops* in the arsenic trioxide tank were more active as compared to those in control tank on the first day of incubation. For an example, *P. sphenops* showed very high movement of pectoral fin and tail fin in experimental

tank containing 1 mg/L of As_2O_3 as compared to control fishes. Their hyperactive behavior was significant in the first week which later on dwindled. In addition to hyperactive behavior of fingerlings, they were not able to recognize the feed when it was added. With the time incubation feed was properly recognized by the fingerlings in dose tank. The behavior of the fishes in dose tanks and control tanks was almost similar at the end of 20 days of incubations and thereafter it remained similar till the end of incubations.

In 60 days of incubations, two fingerlings were found dead in each experimental tank containing 1 mg/L of arsenic trioxide, one each after 2 and 9 days of incubations. This is possible due to high toxicity of arsenic trioxide for young ones of *P. sphenops* or due to lack of feed recognition. However none death was observed in control experimental tank (without arsenic trioxide).

Results presented in the Table-1 evidently suggested the diminution in growth performance of *P. sphenops* in experimental tank containing 1 mg/L of arsenic trioxide as compared to control experimental tank (without As_2O_3) such as standard length, body length, snout length, eye diameter etc. These observations are possibly linked with the chronic exposure to inorganic arsenic which can affect multiple aspects of cellular function including proliferation, apoptosis, differentiation and cell transformation[9]. These effects are evidently explained by Chen et al. through generation of reactive oxygen species in presence of arsenic trioxide[10]. In addition, arsenic can affect cell signaling independently of reactive oxygen species generation[9].

Specific growth rate of *P. sphenops* in experimental tank containing 1 mg/L of As_2O_3 and 0 mg/L (Control) was observed to be 1.66% and 2.09% per day, respectively (Table-2). However specific growth rate of *P. sphenops* in experimental tank containing varying initial concentrations of arsenic need to be investigated further to assess the maximum diminution in specific growth rate. Based on forgoing discussion, it suggests that inorganic arsenic has a significant detrimental effect on growth and development of *P. sphenops*. Details comprehensive studies on specific toxicity of arsenic on internal organs and organ systems are highly crucial to understand the fate of arsenic in the experimental animals.

To best of our knowledge, effects of arsenic trioxide on growth and development of *P. sphenops* has not been reported in literature. However effects on other experimental plants and animals are well known such as reduce growth of marine algae, increase mortality of amphipods, marine red algae failed to reproduce sexually and impaired goldfish behavior[3]. Recently, Rana *et al.,* has reported diminution in growth of *P. sphenops* in presence of 50 mg/L of dimethyl phthalate[11]. These results evidently suggested the sensitivity of *P. sphenops* towards inorganic as well as organic pollutants, even though it has been reported to be highly adoptive species.

Table-1: Diminution of growth performance of *P. sphenops* in presence of As_2O_3.

Characteristics*	0 mg/L of As_2O_3 (Control)	1 mg/L of As_2O_3	Diminution (%)
Standard length (mm)	18.9 ± 2.28	18.24 ±1.57	3.49
Body width (mm)	6.48 ± 1.48	6.08 ±1.06	6.17
Caudal penduncle length (mm)	4.36 ± 0.91	4.10 ±0.47	5.96
Caudal penduncle depth (mm)	4.82 ± 1.12	4.72 ±0.96	2.07
Head length (mm)	4.52 ± 1.11	4.40 ±1.19	2.65
Head width (mm)	3.28 ± 0.53	3.10 ±0.41	5.49
Head depth (mm)	4.00 ± 0.91	3.90 ±0.63	2.50
Snout length (mm)	2.76 ± 1.02	2.38 ±0.61	13.77
Eye diameter (mm)	0.80 ± 0.44	0.66 ±0.36	17.50
Postorbital distance (mm)	2.54 ± 0.72	2.58 ±0.41	-1.57
Inter-orbital distance (mm)	3.76 ± 0.96	3.42 ±0.75	9.04
Pre-dorsal distance (mm)	6.90 ± 0.73	6.80 ±0.47	1.45
Post-dorsal distance (mm)	6.68 ± 0.72	6.54 ±0.50	2.10
Pre-pelvic distance (mm)	8.56 ± 1.26	8.22 ±0.82	3.97
Post-pelvic distance (mm)	12.7 ± 1.10	12.24 ±0.80	3.47
Pre-anal distance (mm)	14.2 ± 0.71	14.06 ±0.54	0.99
Post-anal distance (mm)	2.70 ± 0.57	2.50 ±0.33	7.41
Dorsal fin base (mm)	3.34 ± 0.78	3.44 ±0.43	-2.99
Dorsal fin length (mm)	1.66 ± 0.32	1.70 ±0.27	-2.41
Anal fin length (mm)	0.90 ± 0.41	1.10 ±0.10	-22.22
Anal fin base (mm)	1.20 ± 0.34	1.24 ±0.05	-3.33
Pectoral fin length (mm)	2.90 ± 0.54	2.76 ±0.25	4.83
Pelvic fin length (mm)	1.18 ± 0.21	1.23 ±0.05	-3.81
Pectoral ventral distance (mm)	3.34 ± 0.44	3.44 ±0.34	-2.99
Mouth width (mm)	1.60 ± 0.22	1.62 ±0.16	-1.25
Pre-maxilla length (mm)	1.00 ± 0.00	1.00 ±0.00	0.00

Table-2: Growth performances of *P. Sphenops* in experimental tank containing As_2O_3 and fed with pelletized feed for 60 days feeding trials.

Growth performance	Concentration of As_2O_3 (mg/L)	
	1 mg/L	0 mg/L (Control)
Initial length of fishes (mm)	11.5 ± 4	11.5 ± 4
Final length of fishes (mm)	18.2 ± 2	18.9 ± 2
Gain in length (mm)	6.7	7.4
Initial weight of Fishes (mg)	92.5 ± 7	92.5 ± 7
Final weight of fishes (mg)	184.8 ± 20	208.7 ± 9
Gain in weight (mg)	92.3	116.2
Specific growth rate (%/day)	1.66	2.09
Survival rate (%)	80	100
Mortality rate (%)	20	0

Effect of varying initial concentrations of As_2O_3 on P. sphenops under starvation condition: Increase in mortality of *P. sphenops* in experimental tank was observed with the increase in the concentrations of As_2O_3 (Figure 2). Deaths probit were computed using Finney methods and plotted against logarithmic of As_2O_3 concentrations to calculate lethal concentrations (Figure-3). The LC_{50} value of As_2O_3 for *P. sphenops* was estimated to be 49.5 mg/L. To best of our knowledge, LC_{50} of As_2O_3 on *P. sphenops* has not been reported in the literature. However, LC_{50} values of As_2O_3 for *Cirrhina mrigala*, *Catla catla, Ctenopharyngodon idella*, *Cyprinus carpio*, *Clarias batrachus*, *Labeo rohita*, *Fundulus similis* and *Channa punctatus* were reported to be 24.5, 10.16, 22.17, 32, 84, 28.3, >30 and 10.8 mg/L, respectively[12–18].

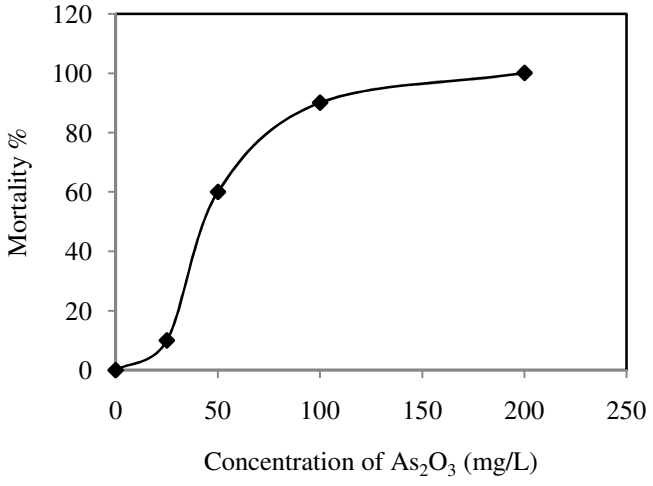

Figure-2: Mortalities of *P. Sphenops* exposed to different concentrations of As_2O_3.

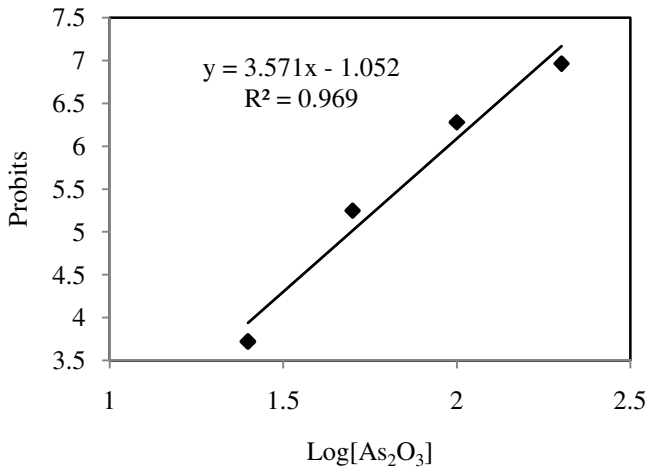

Figure-3: Probits at different concentrations of As_2O_3 for calculation of LC_{50}.

The differences in the value of LC_{50} and overall toxic effect were often reported in the literature for different species and even for same species (Table-3). Difference in LC_{50} is possible due to several factors including differences in the test species, age, feeding habit, sex, composition of toxicant and also the experimental conditions under which the tests are performed. Based on current finding, it is evident that *P. sphenops* is highly sensitive towards As_2O_3. However, effect of other inorganic form of arsenic such as arsenic trihydride, arsenic sulfide, etc. on *P. sphenops* need to be investigated further.

Table-3: Toxicity of Arsenic Trioxide (LC_{50}) on some aquatic organism.

Species	Size/ Age	Duration (h)	LC_{50} (mg/ L)	Ref.
P. sphenop	Finger-lings	96	49.5	Current Study
Cirrhina mrigala	Adult	96	24.5	12
Catla catla	Adult	96	10.16	12
Ctenopharyngodon idella	Adult	96	22.17	12
Labeo rohita	Adult	96	30	12
Cyprinus carpio	Adult	96	32	13
Catla catla	Finger-lings	96	20.41	14
Clarias batrachus	Adult	48	84	15
Labeo rohita	Juvenile	96	28.3	16
Fundulus similis	Juvenile	96	>30	17
Channa punctatus	Finger-lings	96	10.8	18

Conclusion

In current investigations, lethal concentration (LC_{50}) of As_2O_3 on *P. Sphenops* was investigated and found to be 49.5mg/L under experimental conditions. The change in growth performance, morphometric and macroscopic observations evidently indicates the toxicity of As_2O_3 towards *P. Sphenops*. Specific growth rate of *P. sphenops* in experimental tank containing 1 mg/L of As_2O_3 was observed to be 1.66% per day. However in case of control experiment about 2.09% per day was observed. These observations clearly indicate that As_2O_3 has a significant detrimental effect on growth and development of *P. sphenops*. Effect of As_2O_3 on internal organ system such as gonads, liver, brain, etc. need to be investigated further to understand the specific toxic effect.

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