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RESEARCH ARTICLE

TOXICOKINETIC PATTERN OF ARSENIC (As)IN WHOLE BODY AND TISSUES OF *Danio rerio* EXPOSED TO As(III) OXIDE

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ABSTRACT

The bioaccumulation pattern of arsenic (As) in whole body and different tissues of gill, liver, and gonads of both male and female of healthy *Danio rerio* when exposed to a sub lethal concentration of As-water, containing one-eighth 96 hr LC_{50}level (1.11 mg/L As_2O_3.) for long term experimentation has been analyzed. The As shows a maximum deposition ($p<0.05$) in the gill (0.91 and 0.98 µ g / g dry wt.) followed by liver (0.63 and 0.69 µ g / g dry wt.) and gonad (0.21 and 0.27 µ g / g dry wt.) of both male and female fish respectively,at the end of 60 days of exposure. The highest deposition of As was found to be 3.23 and 3.56 µg / g dry wt. in the whole body of male and female fish at 60th day of exposure respectively. Comparing the accumulation As on the both sex, it is obvious that the female showed a higher As residue in all tissue as well as whole body than the male fish. Another equally important finding is that the depuration of As by maintaining the bioaccumulated female fish (60 day exposed fish to As III oxide) in quality dechlorinated tap water reveals that there is a significant ($p<0.05$) reduction in As concentration in different tissues and whole body of fish as the day passes. A comparison of the performance of different tissues in respect of depuration clearly indicates that the liver and gill have taken a short period of 7 days to depurate 100% of As Whereas in ovary and whole body only the level of 81.48 and 94.10 % of As was eliminated even it took 10 days of depuration period. Among the various tissues tested, the ovary did not totally eliminate the accumulated As even after the completion of 10 days of depuration. The data constitute a reference for future studies on the evaluation of As accumulation and elimination tendency in the ecotoxicological testing scheme for hazard assessment.

INTRODUCTION

There is abundant information about arsenic accumulation in aquatic animals in laboratory studies and field aquatic ecosystems that are contaminated by arsenic. Arsenic exists in different chemical forms and oxidation states which influence its bioavailability and toxicity (Agusa *et al.*, 2008). Since arsenic is the dominant chemical in aerobic water and the arsenic was studied in the current project, the arsenic residue information from laboratory studies that tested the accumulation when fish were exposed to arsenic trioxide which was reported in early studies (Cockell *et al.*, 1992; Pedlar *et al.*, 2002b; Suhendrayatna *et al.*, 2001a, 2002). As seen in early reports, there were many species tested for arsenic accumulation, with the arsenic chemical tested. The comparison among these species regarding arsenic accumulation is not practical since different exposure conditions, animal stages and presentation methods for arsenic residue were used in these studies. However, some information could be extracted from these reports. Rainbow trout *Oncorhynchus mykisis* were used in a series of studies at their different developmental stages exposed in different concentration (1.5, 2.0, 3.6, 5.7, 7.6, 13.8, and 48g/l of Arsenic). Different exposure routes were tested, including food and water. In both exposure routes, it was found that arsenic accumulation was dose and time dependent. Most of the arsenicals compounds in the aquatic environment enter into the body through gill respiration and contaminated food. The effect of exposure temperature on arsenic residue levels was organ-specific and depended on arsenic concentration in water. The arsenic levels in whole body rainbow trout exposed to 120 mg/L sodium arsenate at 5^0 C was similar to that at 15^0 C. In contrast, in liver and intestine, arsenic residue was enhanced with temperature (10, 20, and 30^0C), when rainbow trout were exposed to 30 mg/L arsenate. However, when arsenic level increased from 30 mg/L to 60 mg/L, arsenic residue levels were reduced over the temperature range (10, 20 and 30^0C). There are a variety of tissues studied for their capability to accumulate arsenic in laboratory studies. Generally gallbladder plus bile and liver accumulated high concentrations of arsenic, whereas gonad, brain, and bone were among those tissues that did not accumulate arsenic. In general, inorganic arsenic is not an easily accumulated metal. Brown trout (*Salmo trutta*) collected from a contaminated site on the Clark Fork River had significantly higher concentrations of arsenic in gill, liver, kidney and pyloric caeca compared to brown trout collected from a reference site (Farag *et al.*, 1995). In fish, gills are the

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primary sites of respiratory gas exchanges and absorption of pollutants (Garg*et al.,* 2009), while the liver is considered the major organ for metabolization and toxicity of arsenic (Thomas *et al.,* 2001). The accumulation of different arsenic forms mostly depends on the analyzed species and tissue (Fattorini *et al.,* 2004). Many authors have reported that the accumulation of arsenic was higher in marine fish when compared to freshwater fish due to lower concentration of arsenic in freshwater environment (Ferguson and Gavis, 1972; Oladimeji *et al.,* 1984; Norin *et al.,* 1985; Santa Maria *et al.,* 1986). Not much information is available for arsenic depuration characteristies. Uptake and elimination are two of the most important factors in metal mechanisms, but the majority of studies have concerned only on uptake. The elimination routes of metals from fish are generally bile, urine, gill and mucous (Riisgard *et al.,* 1985). Fish depurate rapidly an unaltered chemical like arsenic (As), residue will not accumulate for long-term. It was found that 90% of arsenic residue was eliminated from tilapia *Oreochromis mossambica* and Japanese Medaka *Oryzias latipes*1 day after they were transferred from 1 mg/L arsenite (exposure period: 7days) to arsenic-free water (Suhendrayatna *et al.,* 2001a, 2002). From the above information, the detailed studies are needed to assess the toxicokinetic characteristic of As in whole body and different tissues in test fish of zebrafish and its impact on aquatic organisms.

MATERILAS AND METHODS

Experimental fish

Healthy adult wild-type zebrafish *Danio rerio* 4±1 cm in average length and approximate weight of 0.78 ±0.05g of both male and female fish were purchased from Red hills fish farm, Chennai, Tamilnadu. Fishes were separately maintained at 25±1ºC in 150 capacity glass tank with continuously aerated and dechlorinated tap water (pH 7.1-7.3; hardness 185-200 mg/L as $CaCo_3$; alkalinity 165-170 mg/L as $CaCo_3$) at least one month perior to the experiments. The laboratory photoperiod was 10 hr D; 14 hr L. Fishes were fed with goldfish flake food (or) frozen brine shrimp twice per day *ad libitum.* During routine husbandary, $^1/_3$water was renewed every day with filtered tap water. During the accumulation/ depuration experiments, the fishes were fed with frozen brine shrimp once a day for 1 hr before renewal of test water, after 1 hr, the remaining food was removed.

Exposure chemical

Inorganic Arsenic (As) in the form of As(III)oxide (As_2O_3Analar grade, Hi Media Ltd.,) was used in the present study. The 96 hr LC_{50} concentration of As (III) oxide was 8.91 mg/L for adult zebrafish as calculated by using the Probit analysis method (Finney, 1971). The adult *Danio rerio* was exposed to one–eighth of 96 hr LC_{50} concentration (1.1 mg/L) of As(III)oxide for 60 days to assess the toxicokinetic in the present experiment.

Accumulation Experiment

The accumulation experiment was carried out in glass aquarium (100 L water capacity) wit six replication (30 fish in each exposure). No As(III)oxide was put into aquarium containing the control fish. The water in the control and As_2O_3containing aquarium was renewed every day in order to minimize decrease in the As_2O_3 concentrations. At each interval, after 15^{th},30^{th},45^{th} and 60^{th} days of exposure, six fish were sampled from each group for determination of As in their different tissues. As analysis at every sampling time, the fishes of both sex from control and exposed groups were caught in small net, then rinsed in clean water, and dissected to separate the organ like gills, liver, kidney and gonads of both sexes. To determine the quantity of the accumulated As in various organs and whole body of both sex, the tissues and whole body were placed in separate Petri dishes to dry at 80ºC until reaching a constant weight. The dried materials were powdered separately using a morter and pestle. 100 mg of powdered sample from each tissue was digested separately with a mixture of nitric acid and perchloric acid in the ratio 4:1 and was heated until the sample was almost dry and colourless. The final products were made upto 25ml with double distilled water and the concentration of As was analysed using a Perkin Elmer 3100, an atomic Adsorption Spectrophotometer (ASS). Blanks were also prepared in the same manner as those for the samples. The results were calculated in microgram per gram dry weight.

Depuration Experiment

The determination of depuration period for As-accumulated female fish *Danio rerio* was carried out with remaining 50 fishes, after being exposed to the sublethal concentration (one-eighth of 96 hr LC_{50}) of As(III)oxide for 60 days. Fishes were separately transferred to dechlorinated control water and were allowed to leach accumulated metal from different tissues of the body. Six fish were sacrificed at various intervals for the determination of the As content in the respective sample (tissues). The for Arsenic (As) analysis in tissue and whole body of the fish similar to that described in the bioaccumulation part.

Statistical analysis

Statistical analysis of dada was carried out with one way Analysis OfVariance (ANOVA) and ranked by Duncans multiple range test (Bruning and Kintz, 1968) to compare the data among various tissues studied between treatments for analyzing the significance at 1 and 5 % level.

RESULTS

Bioaccumulation of Arsenic (As) in tissue

The level of Arsenic (As) concentration in the various tissues of the control sample of both sex groups of *Danio rerio* was not detectable in the present investigation. An estimation of the levels of As in different tissues of the male and female fish of *Danio rerio* exposed to a sublethal cum safe level concentration of 1.11mg/L of As over 60 days exposure is presented in Tables 1 and 2 and reveals that the male fish shows the maximum amount of accumulation was found to be 3.23 µg/g dry wt in the whole body and minimum was 0.21µg/g dry wt in the gonad (testis) over 60 days of exposure. The same is true in the female fish also (whole body, 3.56; gonad (ovary), 0.27µg/g dry wt).Organ-wise distribution of residual As (Table 1 and 2) reveals that the gill is the prime site of accumulation followed by liver and gonad in both sexes of test fish.

Table 1. Uptake trend of As(µg/g dry wt) in male *Danio rerio* exposed to 1.11mg/L of As(III) oxide during long-term (60 days) exposure

Sample	Exposure group	Exposure Duration(day)				Significant level
		15	30	45	60	F-value
Whole body	Control	ND	ND	ND	ND	
	Treated	1.63 ±0.03*	1.89± 0.03*	2.22±0.02*	3.23± 0.03	2148.11*
Gill	Control	ND	ND	ND	ND	
	Treated	0.43 ± 0.02	0.58± 0.03	0.72±0.03	0.91±0.02	315.66*
Liver	Control	ND	ND	ND	ND	
	Treated	0.22 ± 0.01	0.36± 0.02	0.51±0.02	0.63±0.03	428.00*
Testis	Control	ND	ND	ND	ND	563.41*
	Treated	ND	0.09± 0.001	0.16±0.01	0.21 ± 0.01	

Values are expressed as mean of six individual ± S.D* F-value ≤ 0.05 => significant at 5 % level between periods of exposure within the same treatment ND - Not detected

Table 2. Uptake trend of As(µg/g dry wt) in female *Danio rerio* exposed to 1.11mg/L of As(III) oxide during long-term (60 days) exposure

Sample	Exposure group	Exposure Duration(day)				Significant level
		15	30	45	60	F-value
Whole body	Control	ND	ND	ND	ND	
	Treated	1.81 ± 0.02	2.23± 0.03	2.68 ± 0.02	3.56 ± 0.04	3451.50*
Gill	Control	ND	ND	ND	ND	
	Treated	0.48 ± 0.01	0.61 ± 0.02	0.79 ±0.03	0.98 ± 0.03	632.14*
Liver	Control	ND	ND	ND	ND	
	Treated	0.26 ± 0.01	0.40 ± 0.01	0.56 ±0.02	0.69 ± 0.02	453.12*
Ovary	Control	ND	ND	ND	ND	2.46*
	Treated	ND	0.12 ± 0.01	0.19 ±0.01	0.27 ± 0.01	

Values are expressed as mean of six individual ± S.D* F-value ≤ 0.05 => significant at 5 % level between periods of exposure within the same treatment ND - Not detected

The gonad of both sexes always contained a significantly lower level of As than any other tissue sampled during the experimentation. Further, the As concentration in whole body and tested tissue of both sexes was gradually increased upto 60 days. As concentration in the testis of male and ovary of female was found to be 0.21 and 0.27 µg/g dry mass respectively at the end of experimentation (60 day). These values are very low in male and female as compared to whole body as well as other organs accumulation at the same period (60 days) of sampling. As accumulation was found to be more in all the tissues of female fish than in male fish during 60 days exposure. The present results reveal a dose and time dependent arsenic accumulation in the different tissue of fishes exposed to sub lethal concentration of arsenic (III) oxide to the different periods of exposure.

Depuration of As from tissue

Periods of depuration estimated for As to various tissues (whole body, gill, liver and ovary) of female fish of As accumulated *D.rerio* (after being exposed to their respective sublethal concentration ($1/8^{th}$ of 96hr LC_{50}) of As over 60 days) are provided in Table 3. As seen from Table 3 whole body and ovary of fish recorded a level of >0.21 and >0.05 µg/g dry wt, respectively, after 10 days of depuration period. The gill and liver of female fish reached the level of 0.10 and 0.08 µg/g dry wt of As respectively, after depuration for a period of 7 days. A comparison of the period of depuration for As in various tissues for short term (10 day) experiments reveals that the gill and liver took minimum number of days for complete recovery to reach the normal levels followed by ovary and whole body of fish. The comparison of the periods of As depuration showed, the ovary (gonad) took more number of days to eliminate the As.

DISCUSSION

Accumulation and occurrence of arsenic was widely reported in plants and animals (Suhendrayatna, 2002; Pedlar *et al.*, 2002a, 2002b). Avariety of fish species, including marine fish, shellfish, and freshwater fish, were found to retain arsenic up to 125.9µg/g dry weight (Koch *et al.*, 2001). Yet, there are no comparative studies between the tissue and whole body accumulation of waterborne arsenic in fish in chronic exposure studies. In a study using a crustacean species, red crayfish *Procambarus calarkii*, as a model animal exposed to Monosodium Methanearsonate (MSMA) herbicide, a dose-dependent arsenic accumulation was observed at arsenic concentration up to 50 mg/L at the intervals of week 2, 4, 6, and 8 and up to 10.93 µg/g wet weight arsenic was found at the 50 mg/L treatment (Naqvi *et al.*, 1990). Furthermore, the authors found that at the 5 mg/L MSMA treatment the crayfish arsenic accumulation is time-dependent, whereas at the lower (0.5 mg/L) and higher concentration (50 mg/L) arsenic residue reached the highest point at the interval of week 4 and 6, respectively (Naqvi *et al.*, 1990). In the current study, the arsenic accumulation in the different tissue and whole body of zebrafish showed time-dependent fashion at a sublethal concentration during chronic exposure. As seen from the Tables 1 and 2 the arsenic residue at a sublethal concentration demonstrated a low rate of accumulation at the initial intervals of 15 and 30 days whereas at 45 and 60 days intervals, the arsenic residue reached the highest point. It indicated that aquatic animals initially can compensate arsenic toxicity to some extent before the critical in tissue as well as whole-body concentrations are reached. Based on a series of studies, McGeachy and Dixon (1989, 1990, 1992) proposed that intoxication or death would occur if a whole- body burden of

arsenic is over 8 µg/g dw, while 4 to 6 µg/gdw is associated with chronic toxicity to fish. Below 2 – 3 µg/g dw results in no mortality in chronic exposure to arsenic in fish. In the current study, the arsenic residue in whole body was in the range of 1.63 to 3.56µ g/g dry weight (Table1 and 2) under chronic exposure. However, most of the arsenic residues fell below 3.5 µg/g dw in both sex at all interval of exposure. Therefore, if zebrafish are similar to those species in the studies by McGeachyand Dixon (1989)in terms of arsenic toxicity, biochemical alterations are expected to occur at these exposure scenarios. No mortality occurred in the current study, which is in agreement with McGeachy and Dixon (1989, 1990, 1992).

'Similar to what has been found in the current study, the time-dependent arsenic uptake in fish has been widely reported in other species. When freshwater fish Tilapia *Oreochromis mossambica* were exposed to arsenic at concentration of 0.1, 5, and 10 mg/L for 7 days, the whole body arsenic residues were 3.4, 7.6, and 11.2 µg/g dw, respectively (Suhendrayatna *et al.*, 2002). However, the arsenic concentrations in fish did not proportionally increase with the increase of the exposure period. In the current study, a significant time-dependent arsenic accumulation was observed. The arsenic residue in the current study at the 1.11 mg/L arsenic treatment at day 60 was only 3.23 µg/g dw in male and 3.56 µg/g dw in female, which were far below that in the *Tilapia* exposure study. These apparent discrepancies can be explained by the species difference because 3 to 10 times difference in arsenic residue was found between *Tilapia Oreochromis mossambica*, Japanese Medaka *Oryzias latipes* and zebrafish in current study in similar exposures. The variation of temperature involved in uptake of chemicals by fish during the test period (McGeachy and Dixon, 1992). Whole body arsenic residue in fish exposed to dietary arsenic showed arsenate doses for 8, 12, and 16 weeks showed dose and time-dependent fashion with respect to whole body of arsenic residue, but roughly equal or higher ration of arsenic residues among the various intervals of exposure corresponding to the arsenic levels in the diets were observed only at 16 weeks (Cockell and Hilton, 1988; Cockell *et al.*, 1991).

Arsenic toxicity depends on its form, and arsenate is usually thought more toxic than arsenate (Aposhian *et al.*, 2004). This is due to their different propensity in uptake relative to animals. Whole body residue of arsenic in 5 mg/L water-borne arsenate was about 2.7 times of that in the same concentration of waterborne arsenate although only 1.07 times was observed in 10 mg/L concentration (Suhendrayatna *et al.*, 2002). Similar observations in another fish species, Japanese Medaka *Oryzia slatipes*, indicated that fish can accumulate more As(III) than As(V) (Suhendryatna *et al.*, 2001a). Significant accumulation occurred at post exposure period of 45 and 60 day in both sexes at sublethal level of arsenic treatments (Table1 and 2). Otherwise at the initial period (15day) of exposure the level of arsenic residue in whole fish body was maintained in low level. This indicated that zebrafish can actively equilibrate arsenic residue in the body at a sublethal arsenic concentrations for short time (15 days) under the continuous exposure, but this equilibrating capability was not efficient if long time exposure (45 and 60 days). Arsenic accumulation is a function of uptake and clearance rates of arsenic in tissues. In order to compensate arsenic toxicity, increasing transformation of arsenic to excretion products and excretion rates, at least, are necessary events to occur. It was found that inorganic arsenics were the predominant arsenicals in Tilapia *Oreochromis mossambica*, with similar contents of As(III) and As(V) when they were exposed to water-borne arsenate upto 10 mg/L and arsenite upto 15mg/L, for 7 days (Suhendryatna *et al.*, 2002). It seems that in short time the biotransformation mechanism contributed little for zebrafish to compensate arsenic toxicity. Improved excretory efficiency of arsenic has been suggested to be responsible for the decreased accumulation of arsenic in fish (Pedlerand Klaverkamp, 2002). Arsenic excretion was reported to be mainly through urine, gill and biliary-fecal excretion routes, and the latter two were found as the major routes in fish exposed to dietary arsenic for 7 days (Oladimeji *et al.*, 1984). The study on arsenic excretion in fish through these routes would gave explanation for the arsenic accumulation pattern observed in the current study.

As seen in Table1 and 2, capability of As accumulation in tissues and whole body of female fish was higher than that in the male fish at all uptake intervals of arsenic treatment.UNEP/FAO (1986) found that the tested heavy metals accumulated more in female crustacean *Nephrops norvegicus* than in male. It was also reported that sex is an important factor to influence the metal accumulation, which lends support to the present finding. On the contrary, Yilmaz and Yilmaz (2007) found that the level of heavy metals in male shrimps was higher than that of females. In the field study, male and female *Gambusia holbrooki* were found to have differing concentration of each heavy metal tested (Van den Broek *et al.*, 2002). Further, they have suggested that this trend of difference in accumulation is related to the physiological differences in sex among fish. From other research, it is clear that the gender-related effects occur in various fishes exposed to contaminants (Burger *et al.*, 2004; Pyle *et al.*, 2005). Gunderson *et al.* (2001) also suggested that the fish exposed to environmental chemicals exhibited gender-specific differences in hepatic metabolism.In the current investigation the mean rate of as As accumulation in the various tissues studied was in the order of gill >liver > gonads > in both sexes (Table 1 and 2). In general, different tissues showed different capacities for accumulation of As.The highest arsenic concentration was found in the gills of both sexes of *Danio rerio* under chronic exposure (60 days) compared to the control gills. The pattern of As accumulation also showed that liver is the main target organ for As next to the gill. Similarly, concentration of As in liver, kidney, spleen and gill of green sunfish after 6 days of exposure were 47.7, 14.2, 18.9 and 3.8 µg/g ww respectively (Sorenson *et al.*, 1979a), whereas concentration of As in liver, kidney, spleen and gill of lake whitefish after 30 days of exposure to 100 µg As/g food were 4.4, 0.76, 0.76 and 0.64 µg/g ww respectively. In contrast to the present finding, the pattern of metal accumulation to various fish species was liver > gill > kidney > gonads observed in experimental (Youssef and Tayel, 2004) as well as in field study (Karadede and Unlu, 2000).

The present results indicated that the accumulation of arsenic in tissues was increased with increasing time of exposure. Similar trend of arsenic accumulation was noted by Hallare *et al.* (2005) in test animal of zebrafish. Cockell *et al.* (1991), Cockell *et al.* (1992) and Cockell and Bettger, (1993) also

found increased arsenic concentrations in liver and kidney of rainbow trout. The accumulation of arsenic in the gill may reflect the excretory roles of these organs (Oladimeji *et al.*, 1984). A maximum concentration level of arsenic accumulation was observed in the gill when compared to other organs of fish exposed to sublethal concentration of As_2O_3 over 60 days of exposure. It shows that gill is the prime site for arsenic accumulation. The reason for this is gill's external position, the highly branched structural organization and resultant highly increased surface area along with the large volume of water passing through the gill surface to make the gill as a prime site for arsenic/metalaccumulation (Mayer *et al.*, 1991; Subathra and Karuppasamy, 2007).

as storage (Odzak and Zvonaric, 1995), would therefore differ from the concentrations detected in the gills and gonads. During long term exposure, toxicants may accumulate in various tissue and leads to changes in the physiological activities. Increased level of arsenic in gonads, attributed to the impairment of gonads, in turn affected the reproduction of *Danio rerio*, which is evidenced in our early finding. Further, our findings are in close agreement with the results of James *et al*. (2003); Karuppasamy (2004); Subathra and Karuppasamy (2007); Puvaneswari and Karuppasamy (2008), who have reported that the accumulation of metal in the test fish has linearly increased with the increasing of exposure period under long-term experimentation.

Table 3. Depuration trend of accumulated As from 60 days of arsenic exposed zebra fish after transferred to chemical free water at various intervals of exposure

Sample	Depuration of As		Depuration rates	Significant level (Between exposure groups)
	Depuration period (day)	As level (µg/g dw)	% change over control	F value
Whole body	0	3.56 ± 0.04		
	3	2.42 ± 0.03	32.02	
	5	1.73 ± 0.02	51.40	
	7	1.13 ± 0.02	68.25	857.53**
	10	0.71 ± 0.03	80.05	
		>0.21 ± 0.01	94.10	
Gill	0	0.98 ± 0.03		
	1	0.88 ± 0.02	10.20	
	3	0.43 ± 0.02	56.12	
	5	0.26 ± 0.03	73.46	943.51**
	7	0.10 ± 0.02	89.79	
	10	ND	100	
Liver	0	0.69 ± 0.02		
	1	0.51 ± 0.02	26.08	
	3	0.30 ± 0.01	56.52	
	5	0.21 ± 0.02	69.56	468.33**
	7	0.08 ± 0.01	88.40	
	10	ND	100	
Ovary	0	0.27 ± 0.01		
	1	0.21 ± 0.01	22.22	
	3	0.16 ± 0.01	40.74	
	5	0.13 ± 0.01	51.85	3.211*
	7	0.09 ± 0.01	66.66	
	10	>0.05± 0.01	81.48	

* F-value ≤ 0.05 => significant at 5 % level** F -value ≤ 0.01 => significant at 1 % level
0 day: values indicate the total uptakes level of As after 60 days exposed zebra fish to 1.11 mg/l As III oxide

The liver is a major site of arsenic accumulation and has been found to readily bind with As. Arsenic accumulation in lake Whitefish liver also reflects the essential role of this organ plays in detoxification (Sorenson, 1991). Different fish species may have differential capability to accumulate arsenic. In fish, liver act as important organ for uptake, accumulation and excretion of arsenic during sublethal treatment (Sorenson, 1991; Maher *et al.*, 1999; Pedlar and Klaverkamp, 2002) (or) other metal like Cu (Subathra and Karuppasamy, 2007). The difference in the levels of accumulation in the different organs/ tissues of fish is primarily attributed to the difference in the physiological role of each organ (Senthilmurugan *et al.*, 2008; Kotze, 1997). Regulatory ability, behavior, feeding habits and other abiotic factors could influence the accumulation differences in the different organs (Karadede and Unlu, 2000; Canli and Atli, 2003; Karuppasamy, 2004; Subathra and Karuppasamy, 2007). The metal concentration in the liver (not in direct contact with the metal in the water), which plays a major role in detoxication (George and Olsson, 1994) as well

Depuration

Depuration studies carried out with *Danio rerio* using As(III)oxide by maintaining the bioaccumulated (for 60 days) test fish after being transferred to quality dechlorinated ground water (As-free water) reveal that there is significant ($p < 0.05$) reduction in metal concentration in whole body and in different tissues as days progressed (Table 3). As seen from Table 3, whole body and ovary of fish recorded a level of >0.21 and >0.05 µg/g after 10 days, respectively. The gill and liver of female fish reached the declined level of 0.10 and 0.08 µg/g of As after a period of 7 days. It appears that in general, the rate of depuration is a relatively slow process to begin with, which speeds up during the later phase of the experiment and is consistent with the report of Subathra and Karuppasamy (2007) for Cu on *M. vittatus*. For metal elimination, there are more numerous routes than uptake routes. However metal accumulation is more rapid than metal elimination because of the presence of metal binding proteins in tissue (Kargin and

Cogun, 1999). Generally, elimination routes of metals from fish are through gill, bile, urine and mucus (Heath, 1995). Like accumulation, several factors influence the elimination of metal from the tissue of fish, such as metals concentration in the target organ, duration time, temperature, interacting agent, age of fish, metabolic activity of fish, abnormalities of organ and biological half-life of the metal (Woo *et al.*,1993; Kargin,1996; Nielsen and Anterson, 1996; Kim *et al.*, 2004; Subathra and Karuppasamy, 2007). There are few studies that examine the depuration of arsenic from aquatic animals, but these few studies have indicated that arsenic depuration occurs rapidly in fish. For example, tilapia and Japanese Medaka *Oryzias latipes* that were exposed to 1mg/L arsenic for 7 days depurated most about 90 % of arsenic residue1 day after being transferred to As-free water (Suhendrayatna *et al.*, 2001a, 2002). Depuration of arsenic in crustacean seems much slower. From 35% to 70% arsenic accumulated during an 8-weeks exposure at the sodium arsenate concentrations up to 50 mg/L was depurated by the red crayfish at the first two weeks of an 8-weeks depurations course (Naqvi *et al.*, 1990). In the current study, when fish were exposed to 1.11 mg/L arsenic(III)oxide, approximately 10%, 20%, 30%, 50% and 100% of the original arsenic body burden (i.e., the whole body concentration after 60 day of exposure) had been eliminated by 1, 3, 5, 7 and 10 days, respectively. This indicates that the depuration of arsenic at initial period (day1and3) was much slower than that after final depuration periods **(**day 5and 10). In the present investigation, the depuration of As clearly indicates that all the selected tissues have taken a lesser number of days to return to a normal level than the days required for such level of As accumulation. Among the various parts (whole body gill, liver, and ovary) examined for depuration, whole body has taken the maximum number of days (1 to 10) followed by gill (1 to 7), liver (1 to 5) and ovary (1 to 5), respectively. A study by Kalay and Canli (2000), Subathra and Karuppasamy (2007) and Mansouri *et al*. (2011b) reported that the gillis the first organ for quick elimination than liver and muscle or gonad of the fish. In conclusion, the accumulation and depuration of As in *Danio rerio* depend on the organ, concentration, and exposure time.

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