Bull. Environ. Contam. Toxicol. (1998) 61: 129-134

Bulletin of Environmental Contamination and Toxicology

Acute and Chronic Effects of Antimony Chloride ($SbCl_3$) on Tilapia (*Oreochromis mossambicus*) Larvae

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Received: 26 January 1998/Accepted: 4 May 1998

The semiconductor industry has the highest revenue and developmental potential in Taiwan. Pollution caused by this industry is not yet understood. Nevertheless, related health and environmental risks, however, from this type of economic activity certainly need to be considered in any study about environmental protection (Edelman 1990). Previous research has often used human cells (Kuroda et al. 1991; Drobyski et al. 1996; Wey et al. 1997) and small mammals (Yamauchi et al. 1986, 1992; Dieter et al. 1991; Burns and Munson 1993; Burns et al, 1994; Morgan et al. 1995; Omura et al. 1996a, b) as experimental subjects for investigating semiconductor metals, but nothing is known about the effects of these metals on aquatic animals and the environment.

Fish embryos and larvae are generally considered to be the most sensitive to environmental pollution and to be effective bioindicators of water quality (Westernhagen 1988).The objective of the present study is to evaluate the impact of one semiconductor metal on aquatic ecology. In particular, our investigation focuses on the acute (2-d) and chronic (16-d) effects of antimony chloride ($SbCl_3$) on larval tilapia (*Oreochromis mossambicus*).

MATERIALS AND METHODS

Mature adult tilapia from the Tainan Branch of the Taiwan Fisheries Research Institute were reared in circulating freshwater at 26-29°C under a photoperiod of 12-14 hr lighting (Hwang et al. 1995). Fertilized eggs and hatched larvae were incubated under the same condition as above. Larvae were not fed during the 2-d toxicity experiments, but were fed daily with 0.S.I.® Cichlid Flake *ad lib* during the 16-d experiments.

To determine 96-h LC_{50}, three-day-old tilapia larvae were transferred to the Sb media at concentrations of 0 (control), 22, 44, 153, and 197 µM (corresponding to 0, 5, 10, 20, 35 and 45 mg/L), respectively. For each concentration level, 45 larvae were used in two 1 -L containers (25 and 20 larvae). Larvae at this stage do not need to be fed as they obtain their energy and nutrition mainly from the yolk sac that usually disappears 10 days after hatching. Thus, starvation should not be a

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cause of mortality in this study. We used $SbCl_3$ (Aldrich, purity: 99.99%) to prepare a 438 µM (100 mg/L) Sb stock solution and diluted it to target concentrations. The 96-h LC_{50} was estimated, using the probit analysis of SAS (SAS Institute Inc, Cary, NC, USA).

The 2-d and 16-d toxicity experiments were conducted for 3- and 30-day-old larvae. The concentrations for 3-day-old larvae were 0, 3, 16, 78, and 156 µM (corresponding to 0, 0.72, 3.6, 18, and 36 mg/L) which were equivalent to 0, 0.02 x, 0.1 x, 0.5 x, and 1 x LC_{50}, respectively. The concentrations for 30-day-old larvae were 0, 78, 156 and 197 µM (corresponding to 0, l8, 36, and 45 mg/L). There was no water change for 2-d tests. For 16-d tests, $SbCl_3$ solution and control (without $SbCl_3$) were changed every 4 days. There were two 1-liter containers (filled up to 800 ml) for each concentration in all tests, 8 to 15 larvae in each container. Five larvae from each concentration were sampled to measure total body length, yolk length (only for 2-d test in 3-day-old larvae), and body ion concentrations (Na, K, Ca, and Mg). The procedures for these measurements were the same as our previous studies (Hwang et al. 1995, 1996). Each ionic content and body length of each sampled larva was measured individually and the measured value then analyzed statistically as a separate observation. One-way analysis of variance and Fisher's pairwise comparisons were performed to examine the effect of the exposure concentration on 3- and 30-day-old larvae respectively (Minitab v. 11.2, Minitab Inc. 1996).

RESULTS AND DISCUSSION

The 96 hr LC_{50} of $SbCl_3$ for 3-day-old tilapia larvae was estimated to be 156 µM (35.5 mg/L = 0.156 x 228.109). Because all death occurred within the first 48 hours of exposure (Table l), this estimate of 96 hr LC_{50} was the same as 48 hr LC_{50}. This value of 156 µM (35.5 mg/L) was more than 1000 times higher than other well studied heavy metals for this stage of tilapia larvae. For example, the 96 hr LC_{50} of Cd for 3-day-old larvae was reported to be 22 µg/L (Hwang et al. 1995).

Table 1. Cumulative death in each treatment concentration during LC_{50} test.

Exposure duration (h)	Concentration (µM)											
	0	22		44		88		153		197	
0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	1	0	8	5	20	25
48	0	0	0	0	0	1	0	8	6	20	25
72	0	0	0	0	0	1	0	8	6	20	25
96	0	0	0	0	0	1	0	8	6	20	25
dead/total	0/12	0/20	0/25	0/20	0/25	1/20	0/25	8/20	6/25	20/20	25/25

The effects of 2-d and 16-d toxicity on 3- and 30-day-old larvae are summarized in Table 2. Those 3-day-old larvae exposed to 78 and 156 µM for 2 days had a significantly smaller total body length than their control (0 µM). The 16-d toxicity effect on growth was also found in 3-day-old larvae exposed to 3 µM, and in 30-

Table 2. The effects of $SbCl_3$ on 3- and 30-day-old tilapia larvae. Values are mean ± SD. n = 5.

larval age (d)	exposure duration (d)	parameter examined	exposure concentration (μM)					
			0	3	16	78	156	197
3	2	body length (mm)	6.81 ± 0.09	6.86 ± 0.10	6.83 ± 0.10	6.63 ± 0.19*	6.53 ± 0.10*	-
3	2	yolk length (mm)	1.56 ± 0.12	1.59 ± 0.08	1.77 ± 0.04*	1.47 ± 0.22	1.53 ± 0.18	-
3	2	body Na (μg/larva)	15.4 ± 1.5	15.9 ± 1.8	18.1 ± 2.7*	14.7 ± 1.8	15.6 ± 1.5	-
3	2	body K (μg/larva)	17.4 ± 1.1	17.0 ± 1.2	17.8 ± 0.8	15.3 ± 2.0*	15.1 ± 0.9*	-
3	2	body Ca (μg/larva)	17.2 ± 2.8	13.2 ± 2.5*	13.3 ± 3.3*	9.9 ± 3.0*	11.9 ± 1.1*	-
3	2	body Mg (μg/larva)	2.5 ± 0.4	2.3 ± 0.3	2.3 ± 0.3	1.6 ± 0.8*	1.5 ± 0.4*	-
3	16	body length (mm)	8.97 ± 0.11	8.56 ± 0.26*	8.84 ± 0.11	NA	NA	-
3	16	body Na (μg/larva)	54.9 ± 19.6	40.6 ± 3.8	41.8 ± 3.4	NA	NA	-
3	16	body K (μg/larva)	36.3 ± 1.4	31.7 ± 3.5*	35.2 ± 1.6	NA	NA	-
3	16	body Ca (μg/larva)	97.1 ± 7.1	84.7 ± 10.1	91.2 ± 11.2	NA	NA	-
3	16	body Mg (μg/larva)	6.3 ± 0.6	5.5 ± 1.5	5.9 ± 0.7	NA	NA	-
30	2	body length (mm)	11.90 ± 1.40	-	-	11.92 ± 1.07	11.30 ± 1.02	10.98 ± 0.86
30	2	body Na (μg/larva)	49.0 ± 14.3	-	-	47.5 ± 15.5	40.4 ± 8.9	34.9 ± 3.0
30	2	body K (μg/larva)v	57.5 ± 14.5	-	-	52.2 ± 8.8	46.6 ± 4.8	37.4 ± 7.2*
30	2	body Ca (μg/larva)	220.7 ± 58.9	-	-	207.2 ± 39.8	193.6 ± 33.7	167.9 ± 17.5
30	2	body Mg (μg/larva)	16.4 ± 5.4	-	-	14.8 ± 3.4	13.1 ± 3.1	11.0 ± 1.9*
30	16	body length (mm)	13.31 ± 0.21	-	-	11.36 ± 0.71*	11.26 ± 0.63*	11.59 ± 0.28*
30	16	body Na (μg/larva)	72.4 ± 6.6	-	-	59.3 ± 12.4*	47.9 ± 7.0*	51.1 ± 5.7*
30	16	body K (μg/larva)	84.5 ± 5.2	-	-	53.1 ± 11.6*	49.0 ± 9.6*	50.9 ± 6.7*
30	16	body Ca (μg/larva)	347.7 ± 22.7	-	-	223.8 ± 30.8*	221.5 ± 31.2*	232.7 ± 21.0*
30	16	body Mg (μg/larva)	24.4 ± 1.9	-	-	14.2 ± 2.4*	13.4 ± 1.9*	13.4 ± 1.6*

-: not tested NA: not available due to fish death before the end of the test

*: significantly different from the respective control (0 μM) at $P < 0.05$.

day-old larvae to all three levels of Sb tested. The effect of Sb on body calcium content was observed in all four combinations of larvae age and exposure duration. Among them, 3-day-old larvae treated for 2 days and 30-day-old ones for 16 days had significantly lower calcium contents than their respective controls. Lower body potassium and magnesium were found in 3-day-old larvae treated with 78 and 156 µM Sb for 2 days. Whether these low in body ion contents were confounded with the smaller body length remains to be determined. However, this may not be the case since there was no difference in body sodium content among the three groups (control, 78 and 156 µM Sb). We did not have a reasonable explanation for the significance of the high body sodium content in the 3-day-old larvae treated with 16 µM Sb for 2 days. Lower body potassium and magnesium were found again in the 30-day-old larvae treated with 197 µM for 2d. Body length and body ion contents were significantly lower in the 30-day-old larvae treated with Sb for 16 days.

Although the semiconductor industry has become one of the leading industries in almost every developed country, study on the toxicity of the metals used in the production line is scarce. The present study is the first one to examine the effects of antimony on aquatic vertebrates. Kuroda et al. (199 1) reported that both trivalent and pentavalent antimony had DNA-damaging activity in the rec assay in which spores of *Bacillus subtulis* were used. Potassium antimony1 tartrate (PAT), a complex salt complex, was used worldwide in the treatment of parasitic disease (Dieter et al. 1991, Wey et al. 1997). PAT was administered intravanenously to humans at a near lethal total dose of 36 mg/kg (Wey et al. 1997). There were several reports on human exposure to trivalent forms of antimony associated with various problems of the circulatory system and even sudden death (Wey et al. 1997). Wey et al. (1997) demonstrated that PAT disrupted intracellular free calcium handling of neonatal rat cardiac myocytes. Dieter et al. (1991) found that PAT was poorly absorbed and relatively nontoxic orally, whereas intraperitoneal injection administration of the drug caused mortality, body weight decrements and lesions in the liver and kidney at doses about one order of magnitude below those in drinking water. In addition, Dieter et al. (1991) reported that rats were more sensitive than mice to the toxic effects of PAT. In summary, the effect of trivalent antimony on calcium balance deserves further notice and the possible species-specific effects should also be taken into account when conducting similar toxic experiments.

Acknowledgements. This study was financially supported by a National Science Council grant of Taiwan (NSC-862621B001003Z). Thanks are extended to Mr. M. H. Chang for his assistance during this study.

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