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Acute and Chronic Effects of Gallium Chloride ($GaCl_3$) on Tilapia (*Oreochromis mossambicus*) Larvae

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The semiconductor industry has the highest revenue and developmental potential of any commercial venture in Taiwan. The extent of pollution caused by this industry, however, is not yet clear. It is possible, though, to assess and predict the potential risks it has for the environment as well as for human and animal life (Edelman 1990). 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) have generally been used in toxicology studies about semiconductor metals. However, nothing is known of the effects of these metals on aquatic animals and environment.

Fish are an important protein source for humans. Consequently, aquatic pollution impacts humans indirectly through the consumption of fish. It is of great importance to evaluate the effects of pollution on fish both for environmental protection and for socio-economic reasons. Fish embryos and larvae are generally considered to be the most sensitive to environmental pollutants, and, thus, they have been widely used as bio-indicators for water quality evaluation (Westernhagen 1988). Burns et al. (1994) indicated that gallium arsenide, an intermetallic semiconductor material, is immunotoxic. Omura et al. (1996) reported testicular toxicity of gallium arsenide to hamsters. With the confounding effect of arsenide, the toxicity of gallium has never been investigated alone. The objective of the present study is to evaluate the impact of gallium (in gallium chloride, $GaCl_3$) on aquatic organisms. Both acute and chronic toxic effects of gallium on tilapia (*Oreochromis mossambicus*) were examined.

MATERIALS AND METHODS

Tilapia larvae are available year-round from mature adults if conditions are controlled at 26-29°C under a photoperiod of 12-14 h lighting (Hwang et al. 1992). Mature adult tilapia obtained from the Tainan Branch of the Taiwan Fisheries Research Institute were reared in circulating freshwater under the conditions described above. Fertilized eggs and hatched larvae were incubated under the same condition as above. Larvae were not fed during the acute toxicity experiments, but

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were fed daily with O.S.I.® Cichlid Flake during the chronic experiments. To determine 96-h LC_{50}, three-day-old tilapia larvae were transferred to the Ga media at concentrations of 0 (control), 114, 198, 256 and 568 µM, respectively. For each concentration level, eighty larvae were used in two 1-L containers. (40 larvae each). Larvae at this stage do not need to be fed and they obtain their energy and nutrition mainly from the yolk sac that usually disappears 10 days after hatching. And, starvation should not be a cause of their mortality in this study. We used $GaCl_3$ (Aldrich, purity: 99.99%) to prepare a 568 µM (100 mg/L) Ga 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 acute (2-d) and chronic (16-d) toxicity experiments were conducted for 3- and 30-day-old larvae. The concentrations for 3-day-old larvae were 0,4.1, 20.4, 102.4, 204.5 and 255.6 µM which were equivalent to 0, 0.02 x, 0.1 x, 0.5 x, 1 x and 1.25 x LC_{50}, respectively. The concentrations for 30-day-old larvae were 0, 102.4, 204.5 and 255.6 µM. There was no water change for acute tests. For chronic tests, $GaCl_3$ solution and control (without $GaCl_3$) 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 acute test in 3-day-old larvae), body ion contents (Na, K, Ca, and Mg). The procedures for these measurements were the same as our previous studies (Hwang et al. 1995, 1996). One-way analysis of variance and Dunnett test (with the family error rate set at 0.05 to compare with their respective controls) were performed to examine the effect of the exposure concentration on 3- and 30-day-old larvae (Minitab v. 11.2, Minitab Inc. 1996).

RESULTS AND DISCUSSION

The 96 h LC_{50} of $GaCl_3$ for 3-day-old tilapia larvae was estimated to be 204 µM (36 mg/L = 0.204 x 176.079). Because all deaths occurred within the first 48 hours of exposure, this estimate of 96 h LC_{50} is the same as 48 h LC_{50}. This value of 204 µM (36 mg/L) was more than 1000 times higher than other well studied heavy metals for this stage of tilapia larvae. For example, the 96 h LC_{50} of Cd for 3-day-old larvae was reported to be 22 µg/L (Hwang et al. 1995).

The effects of acute and chronic toxicities on 3- and 30-day-old larvae are summarized in Table 1. The 3-day-old larvae exposed to 204 µM (1 x LC_{50}) for 2 days had a significantly shorter total body length and larger yolk diameters than their control (0 µM), indicating a retardation in body growth. This retardation on body growth was also found in the 30-day-old larvae exposed for 16 days. The toxic effects of $GaCl_3$ on ion contents were found to be significant in 3-day-old larvae exposed to 20.4 µM (0.1x LC_{50}) for 2 days and 102.2 µM (0.5x LC_{50}) for 16 days. The acute effects on this stage of larvae were to decrease their body ion contents while the chronic effects to increased this (except for body calcium content). Findings for the chronic toxicity test may partly be due to the smaller

Table 1. The acute and chronic effects of $GaCl_3$ on 3- and 30-day-old tilapia larvae. Values are mean ± SD. n = 5.

age (day) – exposure duration (day)	parameter examined	exposure concentration (μM)					
		0	4.1	20.4	102.2	204.5	255.6
3-2	body length (mm)	6.46 ± 0.11	6.52 ± 0.14	6.51 ± 0.16	6.43 ± 0.16	5.67 ± 0.10*	NA
	yolk length (mm)	1.54 ± 0.09	1.51 ± 0.04	1.51 ± 0.04	1.42 ± 0.07*	1.71 ± 0.06*	NA
	body Na	12.8 ± 1.0	12.9 ± 1.2	9.8 ± 0.8*	10.8 ± 2.3	13.6 ± 1.2	NA
	body K	15.8 ± 0.3	16.3 ± 1.0	10.8 ± 0.4*	15.5 ± 1.0	16.4 ± 0.4	NA
	body Ca	8.4 ± 1.1	7.6 ± 0.7	3.5 ± 0.6*	8.3 ± 1.2	10.0 ± 3.8	NA
	body Mg	1.7 ± 0.3	1.8 ± 0.3	1.2 ± 0.3*	1.6 ± 0.2	1.8 ± 0.3	NA
3-16	body length (mm)	8.30 ± 0.22	8.55 ± 0.17	8.59 ± 0.32	8.86 ± 0.18*	NA	-
	body Na	27.1 ± 1.7	30.6 ± 2.4	30.1 ± 4.3	37.4 ± 6.2*	NA	-
	body K	25.7 ± 1.5	28.3 ± 1.2	27.4 ± 3.0	28.3 ± 1.5	NA	-
	body Ca	70.6 ± 4.5	76.2 ± 5.0	74.7 ± 9.0	61.6 ± 3.6	NA	-
	body Mg	3.9 ± 0.4	4.7 ± 0.3*	4.4 ± 0.7	4.9 ± 0.3*	NA	-
30-2	body length (mm)	10.5 ± 1.1	-	-	9.6 ± 0.6	10.5 ± 1.0	NA
	body Na	88.0 ± 10.5	-	-	79.1 ± 17.7	64.4 ± 15.7	NA
	body K	39.4 ± 2.2	-	-	46.5 ± 16.0	33.9 ± 11.3	NA
	body Ca	157.1 ± 20.3	-	-	173.5 ± 44.6	143.3 ± 18.9	NA
	body Mg	9.6 ± 0.8	-	-	10.8 ± 3.6	8.2 ± 1.9	NA
30-16	body length (mm)	12.5 ± 1.7	-	-	11.1 ± 0.9	11.3 ± 0.6	11.6 ± 0.3
	body Na	72.5 ± 6.6	-	-	59.3 ± 12.4	47.9 ± 7.0*	51.1 ± 5.7*
	body K	84.5 ± 5.1	-	-	53.1 ± 11.6*	49.0 ± 9.6*	50.9 ± 6.7*
	body Ca	347.7 ± 22.7	-	-	223.8 ± 30.8*	221.5 ± 31.2*	232.7 ± 20.9*
	body Mg	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 $P < 0.05$.

size in the control group. The most evident effect of $GaCl_3$ on ion contents was from the 30-day-old larvae exposed to 102.2 - 255.6 µM for 16 days. All of the four body electrolytes were found to be significantly lower than their respective controls.

For the 3-day-old larvae, all 25 fish died within 10 days when they were exposed to 204.5 µM. The concentration became more tolerable and no mortality was found for larvae of 30 days old. This difference in metal tolerance among various developmental stages of tilapia was consistent with our earlier Cd studies (Hwang et al. 1995). However, at a higher concentration of 255.6 µM, all of the five 30-day-old larvae died before the end of the 2-d toxicity test. This implies that there is only a small increase (if at all) in toxicity tolerance from 3-day-old larvae to 30-day-old larvae. There were several larvae left at the end of the 16-d exposure at 255.6 µM and five of them were sampled for the measurements.

Gallium is a group IIIa metal and it binds avidly to the ion transport protein, transferrin and can be incorporated into the iron storage protein, ferritin (Drobyski et al. 1996). The transferrin-gallium complex blocks iron uptake and results in iron deficiency and inhibition of cellular proliferation. Gallium also interferes with calcium uptake. Warrell (1991) applied gallium nitrate to inhibit bone resorption to patients with hypercalcemia. In their studies on the testicular toxicity of GaAs, Omura et al. (1996a, b) found a 32 times higher serum Ga than As concentration in GaAs-treated hamsters and the disturbance of GaAs in the development of sperm formation, Thus, they suggested a more important role for Ga in the testicular toxicity of GaAs in hamsters. Although findings from the present study indicates that the acute toxicity of Ga was not as high as other better-known heavy metals (such as Cd, Cu, Hg, and Zn), its chronic physiological effects might be more important and should be examined.

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