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Acute and Chronic Effects of Indium Chloride ($InCl_3$) on Tilapia (*Oreochromis mossambicus*) Larvae

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Semiconductor production is the industry with the highest revenue and developmental potential in Taiwan. The effect of pollution caused by the semiconductor industry is not yet clear. Nevertheless, related health and environmental risks resulting from semiconductor production are predicted to be an important issue in environmental protection (Edelman 1990). The most common subjects for the toxicology studies on the semiconductor metals (Ge, GaAs, In, Sb) are 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). Nothing is known of the effects of these metals on aquatic animals and the environment.

Fish are an important protein source for humans, and fishing is one of the most important industries in Taiwan. Any pollution in the aquatic environment which impacts physiology, development, growth or survival of fish affects human that, at the top of the food chain, consume fish. Therefore, it is of great importance to evaluate the effects of pollutants on fish for both environmental protection and socio-economic reasons. Fish embryos and larvae are generally considered to be the most sensitive to environmental pollutants, thus they have been widely used as bio-indicators for water quality evaluation (Westernhagen 1988). The objective of the present study is to evaluate the impact of one of the semiconductor metals on the aquatic organisms. Both 2-d and 16-d toxic effects of indium chloride ($InCl_3$) on tilapia *(Oreochromis mossambicus)* were examined.

MATERIALS AND METHODS

Tilapia larvae are available year-round from mature adults if the rearing conditions are controlled at 26-29°C under a photoperiod of 12-l4 hr lighting (Hwang et al. 1995). Under these conditions, mature adult tilapia were reared and fertilized eggs and hatched larvae were incubated. Larvae were not fed during the 2-d toxicity experiments, but were fed daily *ad lib* with 0.S.I.® Cichlid Flake during the 16-d experiments.

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To determine 96-h LC_{50}, three-day-old tilapia larvae were transferred to the In media at concentrations of 0 (control), 136, 158, 181, and 203 µM , respectively (corresponding to 0, 30 35, 40, and 45 mg/L). For each concentration level, fifty larvae were used in two 1 -L containers (25 larvae each). 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 cause of mortality in this study. We used $InCl_3$ (Aldrich, purity: 99.99%) to prepare a 452 µM (100 mg/L) In 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, 17, 85, and 170 µM (corresponding to 0, 0.75, 3.75, 18.75 and 37.5 mg/L) which were equivalent to 0, 0.02, 0.1 x, 0.5x, and 1 x LC_{50}, respectively. The concentrations for 30-day-old larvae were 0, 85, 127, 170 and 213 µM (corresponding to 0, 18.75, 28.1, 37.5 and 46.9 mg/L). 'There was no water change for 2-d tests. For 16-d tests, $InCl_3$ solution and control (without $InCl_3$) were changed every 4 days. There were two 1 -liter containers (tilled 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 sac 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 the body length of each sampled larva were measured individually and the measured value 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

Table 1. Cumulative death during LC_{50} test.

Exposure duration (h)	Concentration (µM)								
	0	136		158		181		203	
0	0	0	0	0	0	0	0	0	0
24	0	0	0	3	4	25	25	25	25
48	0	0	0	3	4	25	25	25	25
72	0	0	0	3	4	25	25	25	25
96	0	0	0	3	4	25	25	25	25
dead/total	0/25	0/25	0/25	3/25	4/25	25/25	25/25	25/25	25/25

The 96 hr LC_{50} of $InCl_3$ for 3-day-old tilapia larvae was estimated to be 170 µM (37.6 mg/L = 0.170 x 221.179). Because all deaths occurred within the first 48 hours of exposure (Table 1), the estimate of 96 hr LC_{50} was the same as 48 hr LC_{50}. This value of 170 µM (37.6 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 2. The effects of $InCl_3$ 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	17	85	127	170
3	2	body length (mm)	6.70 ± 0.13	6.79 ± 0.13	6.90 ± 0.03*	6.97 ± 0.07*	-	6.78 ± 0.13
3	2	yolk sac length (mm)	1.61 ± 0.06	1.62 ± 0.05	1.66 ± 0.05	1.65 ± 0.08	-	1.58 ± 0.28
3	2	body Na (μ g/larva)	16.8 ± 2.5	15.9 ± 0.7	15.6 ± 0.7	16.1 ± 0.8	-	15.3 ± 0.3
3	2	body K (μ g/larva)	16.7 ± 0.8	16.7 ± 0.4	17.8 ± 0.6*	18.3 ± 1.2*	-	17.7 ± 0.8
3	2	body Ca (μ g/larva)	18.4 ± 11.6	15.3 ± 5.2	10.0 ± 1.7	10.6 ± 0.8	-	12.2 ± 6.4
3	2	body Mg (μ g/larva)	2.4 ± 0.4	2.5 ± 0.0	2.6 ± 0.2	2.8 ± 0.3*	-	2.5 ± 0.0
3	16	body length (mm)	9.38 ± 0.19	9.32 ± 0.20	9.26 ± 0.17	8.90 ± 0.21*	-	8.71 ± 0.29*
3	16	body Na (μ g/larva)	47.6 ± 3.2	48.0 ± 4.9	49.2 ± 7.6	32.3 ± 4.9*	-	27.2 ± 2.3*
3	16	body K (μ g/larva)	39.3 ± 1.5	39.8 ± 2.2	42.1 ± 5.4	33.6 ± 1.2*	-	30.3 ± 2.4*
3	16	body Ca (μ g/larva)	95.4 ± 7.3	93.4 ± 10.2	91.3 ± 15.1	58.5 ± 7.6*	-	53.0 ± 5.5*
3	16	body Mg (μ g/larva)	7.2 ± 0.6	6.9 ± 0.8	6.7 ± 1.3	4.4 ± 0.4*	-	3.8 ± 0.6*
30	2	body length (mm)	10.54 ± 1.08	-	-	10.07 ± 0.99	9.91 ± 0.32	9.43 ± 0.36*
30	2	body Na (μ g/larva)	102.6 ± 25.8	-	-	97.6 ± 28.7	100.2 ± 14.0	74.4 ± 8.8*
30	2	body K (μ g/larva)	47.0 ± 16.3	-	-	40.3 ± 16.4	39.9 ± 6.7	28.2 ± 4.4*
30	2	body Ca (μ g/larva)	182.5 ± 42.3	-	-	175.8 ± 33.8	168.5 ± 23.3	149.8 ± 10.7
30	2	body Mg (μ g/larva)	11.4 ± 3.8	-	-	10.5 ± 3.3	10.4 ± 2.1	8.2 ± 0.9
30	16	body length (mm)	9.80 ± 0.30	-	-	9.94 ± 0.65	9.44 ± 0.33	9.64 ± 0.74
30	16	body Na (μ g/larva)	114.4 ± 8.3	-	-	111.4 ± 19.7	86.6 ± 15.2*	95.2 ± 29.4
30	16	body K (μ g/larva)	47.4 ± 4.4	-	-	50.0 ± 13.9	37.8 ± 11.2	42.2 ±13.1
30	16	body Ca (μ g/larva)	170.0 ± 16.2	-	-	165.8 ± 16.4	135.8 ± 9.0*	148.5 ± 26.5
30	16	body Mg (μ g/larva)	10.9 ± 1.5	-	-	11.1 ± 2.4	8.1 ± 1.6*	8.9 ± 2.1

-: not tested

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

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 17 and 85 µM for 2 days had a significantly greater total body length than their control (0 µM). For the rest of three tests (16-d tests on both 3- and 30-day-old larvae and 2-d test on 30-day-old larvae), the effects of $InCl_3$ on fish growth are significant; the higher the treatment concentration, the shorter the total body length. The toxic effects of $InCl_3$ on ion concentrations were significant (1) in 3-day-old larvae exposed to either 85 or 170 µM (0.5 and 1 x LC_{50}) for 16 days, (2) in 30-day-old larvae to 170 µM (1 x LC_{50}) for 2 days and (3) in 30-day-old larvae to 127 µM (1 x LC_{50}) for 16 days. The 30-day-old larvae exposed to 170 µM $InCl_3$ for 16 days had a smaller body size and lower ion contents than their controls. However, the difference was not statistically significant. When the 30-day-old larvae were exposed to 213 µM (1.25 x LC_{50} of 3-day-old larvae), all 25 individuals died (data not shown), indicating that there was no increase in tolerance to indium in the 30- day-old larvae.

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 indium on aquatic vertebrates. There were, however, some studies on the comparative toxicity of indium arsenide and indium oxide to hamsters (Yamauchi et al. 1992, Omura et al. 1996a) and rats (Omura et al. 1996b). Omura et al. (1996b) evaluated the toxicity of one of a binary compound semiconductors, InAs, and found a decrease in sperm count in the epididymis of 12 month-old rats. However, no testicular toxicity was observed in As_2O_3-treated rats, indicating that indium, together with arsenic, is important in the testicular toxicity of InAs in rats. A different serum concentration of indium and arsenic in hamsters receiving repetitive intratracheal instillations of InAs was observed in a similar experimental design by Omura et al. (1996a). The serum concentration of In was 57-times higher than that of As in InAs-treated hamsters. This observation suggests that it may take some time before the toxic effects of In can be found and the metabolism (or excretion) of In was much slower than that of As in the hamsters (Yamaughi et al. 1992). In addition, Yamauchi et al. (1992) found that In and As showed a different accumulation pattern in various tissues of hamsters. Peak concentrations of the arsenic content of various organs were observed within 3 to 5 days following a single subcutaneous InAs administration. On the other hand, indium concentrations of all four organs (liver, kidney, lung and spleen) continued to rise till the end of the 30-day experiment. These results suggested that the possible chronic (16 d) toxic effects of indium should be studied alone; that is, without the effects of As. And, apparently, the toxic effect of indium was species-specific and possibly also stage-specific. This stage-specificity was observed in our study; the chronic effects on body ion contents were found in the 3-day-old larvae but not in the 30-day-old ones when they were treated with 1 x LC_{50} (170 µM) indium. These characters should be taken into account for further studies on the biological toxicity of In.

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