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Growth Retardation and Histopathology of Common Carp (*Cyprinus carpio*) Exposed to Gallium

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Gallium is one of the intermetallic elements which are increasingly being used for the manufacture of semiconductor devices in Taiwan. Gallium compounds such as GaAs and AlGaAs are important materials for the manufacture of integrated circuits and optoelectronic devices in the semiconductor industry (Robinson 1983; Bustamante et al. 1997). Manufacturing processes devoted to the fabrication of GaAs-based semiconductor devices generate large volumes of wastes that contain the toxic metal arsenic as well as gallium. For example, discharged wastewater from the gallium arsenide wet polishing process can contain from 200 to 400 mg L^{-1} of each dissolved metal (Sturgill et al. 2000). However, gallium arsenide is not listed as a hazardous waste under local regulations (in Taiwan), but is listed as hazardous in California in the US (Sturgill et al. 1999). Previous reports demonstrated that gallium compounds may cause pulmonary and testicular toxicity in mammals (Webb et al. 1987; Omura et al. 1996). As to aquatic animals, tilapia larvae (*Oreochromis mossambicus*) also show retardation in body growth with sublethal concentrations of gallium (Lin and Hwang 1998a). Many studies examining the sensitivities of early life stages have revealed that larval stages are the most sensitive to heavy metals and semiconductor metals (Cleveland et al. 1986; Witeska et al. 1995; Lin and Hwang 1998b). Furthermore, histopathological alterations are recognized and commonly used diagnostic tools in fish toxicology studies (Lloyd 1992). There are several studies examining gill and kidney histological alterations induced by heavy metals in aquatic environments. For instance, exposure to copper can damage gill filaments, renal tubules, and glomeruli in common carp (De Boeck et al. 1995; Karan et al. 1998). Adverse effects of exposure to cadmium have also been found in branchial structures of tilapia (Wong and Wong 2000).

Because the common carp is an important cultured fish species in fishponds near semiconductor manufacturing districts in Taiwan, it is a suitable model species to study the toxicity of semiconductor-related metals. Therefore, the purposes of this study were to investigate the effects of sublethal gallium concentrations on growth and histopathological changes in the common carp. The results will provide information which can be used to objectively institute measures to minimize the pollution by gallium and its impacts on aquatic ecosystems.

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MATERIALS AND METHODS

Fry and juveniles of common carp (*Cyprinus carpio*) were obtained from the Chupei Branch of the Taiwan Fisheries Research Institute. Fish were transported to the glass aquarium in our laboratory which was equipped with a water-cycling device; dechlorinated tap water (pH 7.4-7.8; dissolved oxygen concentration 7.3-8.1 mg L^{-1}; hardness 38-45 mg $CaCO_3$ L^{-1}) was used during the entire experiment. The temperature was maintained at 25.0 $\pm$0.5 °C, and the photoperiod was set at 12 h of light and 12 h of dark. Fish were acclimated for 2 weeks and fed aquarium fish mixture twice a day. Fry (4 weeks old, 0.202 ± 0.006 g in body weight) were used for acute toxicity tests and growth tests; juveniles (12 weeks old, 2.3 ± 0.19 g in body weight) were used for acute toxicity tests and histological examinations in the initial experiments. Gallium sulfate (purity 99.999%) was purchased from Alfa Aesar (Ward Hill, MA). A stock solution was prepared in deionized water (1000 mg L^{-1} gallium in 0.1% nitric acid).

Laboratory static renewal tests were conducted to determine the median lethal concentration (LC_{50}) for fry and juvenile carp. Ten fish of similar size were randomly sampled and placed in 20-L glass beakers. After 24 h of acclimatization, fish were exposed to different gallium concentrations (0, 4.0, 8.0, 12.0, 16.0, 20.0, 24.0, and 28.0 mg L^{-1}) for 96 h or more. The control and each treated group were run in duplicate. During the experiment, dead fish were removed, and mortality was recorded after 24, 48, 72, and 96 h. The LC_{50} of gallium and its 95% confidence limits for carp were calculated using a Basic program from the probit analysis described by Finney (1971).

In the growth-inhibition study, groups of 10 fry were randomly sampled and placed in 20-L glass beakers; fish were then exposed to test solutions of 0.0, 1.0, 2.0, and 4.0 mg L^{-1}, respectively in triplicate. Exposure time was 4 weeks. Fish were fed 10% of their body weight with aquarium fish mixture twice daily (at 06:00 and 18:00). The remaining food was removed after half an hour to keep the water clean. Wet body weights were measured using an electrical balance after 7, 14, 21, and 28 days of exposure. In order to minimize the capture stress, fish were anesthetized with MS-222 (Sigma Chemical, St. Louis, MO) at a concentration of 1:8000, and excess water was removed by blotting the fish with a soft tissue. The specific growth rate (SGR) was calculated according to the equation of Brown (1957):

$$SGR\ (\%\ day^{-1}) = 100(\ln W_2 - \ln W_1)/(T_2 - T_1)$$

where W is the weight of the carp (g wet wt) and T is the time (days) at the start (1) and at the end (2) of the growth period.

Juvenile carp for histopathology tests were randomly placed in replicate 50-L glass aquaria. Every tank contained 10 fish which were exposed to test solutions containing 0.0, 1.0, 4.0, and 8.0 mg L^{-1} gallium, respectively. Both control and

treatment fish were taken after 2 weeks of exposure time. Four fish per concentration were anesthetized with MS-222 (1:5000) and then sacrificed. Both the gills and kidneys were collected, fixed in 4% buffered formalin, and processed routinely for examination using standard techniques with hematoxylin and eosin (H&E).

Statistical differences in growth rates at various gallium concentrations were tested by one-way analysis of variance (ANOVA). A level of $p < 0.05$ was determined to be statistically significant.

RESULTS AND DISCUSSION

Median lethal concentrations (LC_{50}) of gallium for fry (4-week-old) and juvenile (12-week-old) carp were obtained. Values for the 48-, 72-, and 96-h LC_{50} are presented in Table 1. It is clear that the higher the concentration, the shorter the LC_{50} of the animals. Fry were also found to be more susceptible than juveniles to acute gallium toxicity. Comparing the toxicity of gallium with those of other metals studied such as copper (96-h LC_{50}: 0.64 mg L^{-1}) and zinc (96-h LC_{50}: 17 mg L^{-1}) for the same species (Karan et al. 1998; Lam et al. 1998), it is clear that the toxicity of gallium is no stronger than that of copper and zinc. Further, the 96-h LC_{50} value of gallium for 3-day-old tilapia larvae (*Oreochromis mossambicus*) was estimated to be 14.32 mg L^{-1} (Lin and Hwang 1998a), indicating that tilapia is more tolerant to gallium exposure than is the common carp.

Table 1. Median lethal concentrations (LC_{50}) of gallium to common carp.

	LC_{50} (mg Ga L^{-1})		
Life stage	48 h	72 h	96 h
Fry	17.80	15.70	12.55
	(16.39-19.33)	(12.37-19.95)	(10.82-14.54)
Juvenile	28.81	22.11	19.78
	(21.92-37.88)	(20.69-23.63)	(18.49-21.16)

The 95% confidence limits are given in parentheses.

Concentrations used in the growth effects of gallium on fry carp were equivalent to approximately 8%, 16%, and 32% of the 96-h LC_{50} value according to our acute toxicity study. Therefore no mortality was recorded during the 4 weeks of the experiment for all exposure concentrations studied. Within the first week of the experiment, no significant differences in growth rates were recorded ($p < 0.05$). However, specific growth rates of fry were significantly reduced from the second week onwards at 2.0 and 4.0 mg Ga L^{-1} ($p < 0.05$) (Fig. 1). At a gallium concentration of 1.0 mg L^{-1}, no inhibition of growth was observed in the present study. Thus, 1.0 mg L^{-1} is proposed as a biologically safe concentration which can be used for establishing tentative water quality criteria.

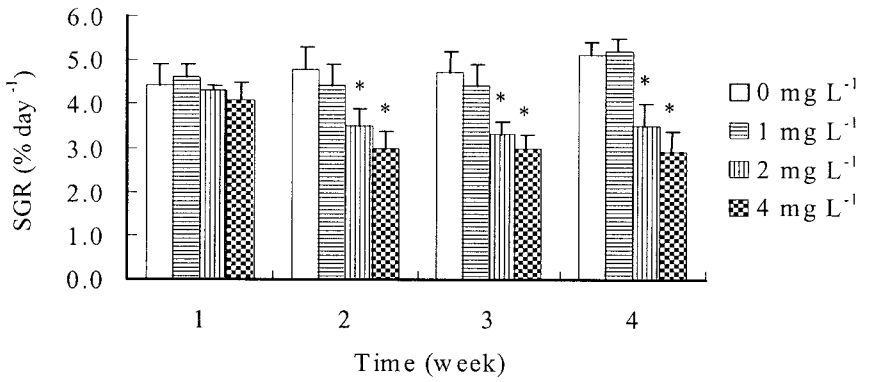

Figure 1. Effect of gallium on the specific growth rate (SGR, % day^{-1}) of fry carp during the experimental period (mean ± SD, n = 30). *Indicates a significant difference compared to the control group ($p < 0.05$).

Reductions in growth rates of carp fry were positively correlated to gallium concentrations at the two highest sublethal levels. Growth depression depends on loss of appetite, toxicant concentration, and physiological adaptations (Lett et al. 1976; De Boeck et al. 1997). There were no differences in feeding activities between the control and any treatment for food consumption tests (data not shown). Thus, reduced growth at the concentrations of 2.0 and 4.0 mg L^{-1} of gallium might not be attributable to loss of appetite; other factors such as increased basal metabolism for detoxification, adaptation, and repair of tissue should also be considered as causes for the slower growth. Further, no retardation in growth was seen at 1.0 mg Ga L^{-1} which is equivalent to 8% of the 96-h LC_{50} value, and is in good agreement with the concept of a safe level (one-tenth of the 96-h LC_{50} value) as described by Sprague (1971).

Gills of juvenile carp from the control group displayed normal morphological structures (Fig. 2). Slight hypertrophy of mucous and chloride cells were found in the 1.0-mg Ga L^{-1} exposure group. Pronounced changes in gills of fish exposed to 4.0 mg Ga L^{-1} including subepithelial edema and proliferation of mucous cells on the bottom of the secondary lamellae were observed (Fig. 3). Similar histopathological changes clearly remained at 8.0 mg Ga L^{-1}, while hyperplasia of the epithelia leading to fusion of the distal ends of lamellae was noted (Fig. 4). Lesions in juvenile carp increased in severity at higher gallium concentrations.

Subepithelial edema and epithelial lifting usually result in increased distances for the exchange of gases and metabolites with water (Ferguson 1989; Mwase et al. 1998). Hypertrophy of chloride cells on the gills can be interpreted as possibly having a detoxification function (Mallatt 1985; McDonld et al. 1991). In addition, gallium can induce an increase in mucous cells on the gills. Ferguson (1989) indicated that mucous sialic acid groups could effectively bind diffusible cations in aquatic environments, which is a typical defense mechanism of fish gills. These

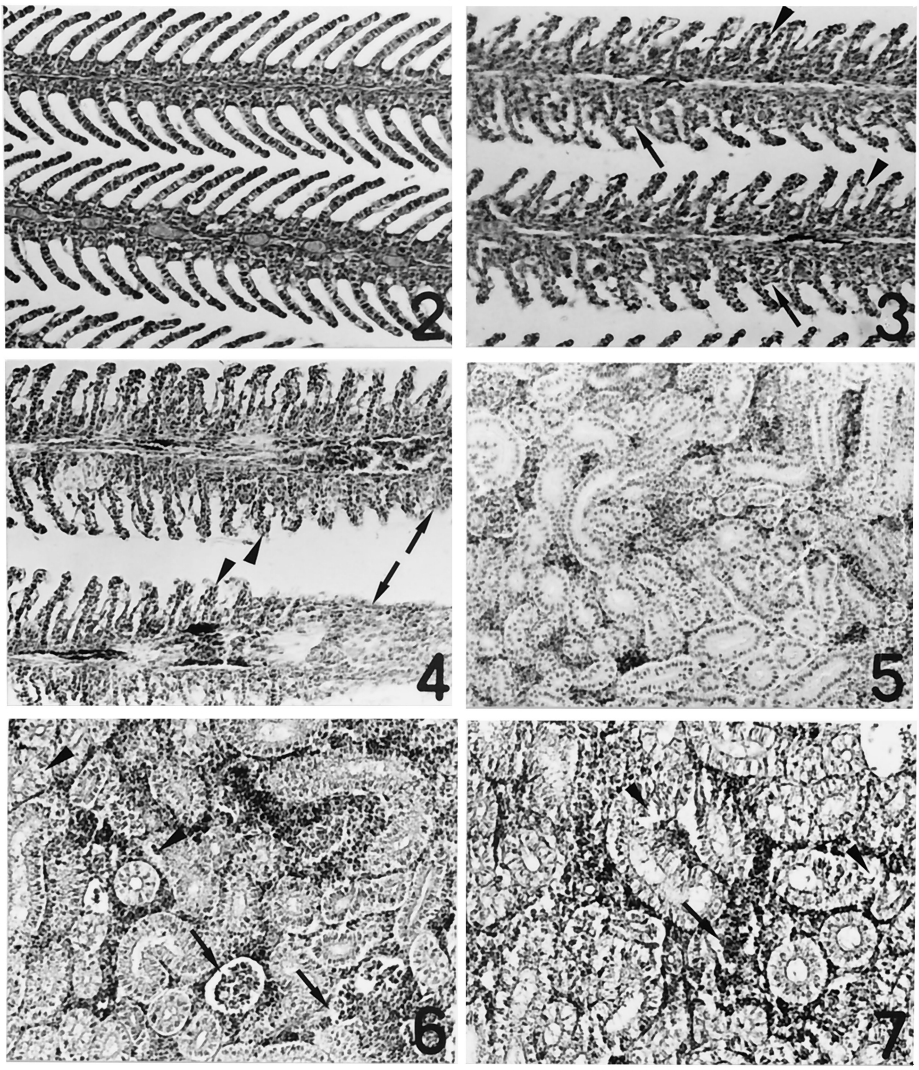

Figure 2. Gill tissue of juvenile carp in the control. **Figure 3.** Gill tissue of juvenile carp exposed to 4.0 mg Ga L^{-1} for 14 days. Note the proliferation of mucous cells on the bottom of the secondary lamellae (arrows); subepithelial edema can also be seen (arrowheads). **Figure 4.** Gill tissue of juvenile carp exposed to 8.0 mg Ga L^{-1} for 14 days. Note the hyperplasia of the epithelia (arrowheads) and fusion of the adjacent lamellae (arrows). **Figure 5.** Renal tissue of juvenile carp in the control. **Figure 6.** Juvenile carp exposed to 4.0 mg Ga L^{-1} for 14 days. Note the hypertrophy of the tubular epithelia (arrowheads), and ectasis of Bowmen's capsules (arrows). **Figure 7.** Juvenile carp exposed to 8.0 mg Ga L^{-1} for 14 days. Note the severe damage to the tubular epithelia (arrowheads), and degenerative changes in the glomeruli (arrow). (H&E stain, original magnification ×200)

lesions have also been reported as sublethal responses to heavy metals such as cadmium, mercury, and copper (Lloyd 1992; Jagoe et al. 1996; Karan et al. 1998; Wong and Wong 2000).

The control group and fish treated with 1.0 mg Ga L^{-1} showed no histopathological changes of the kidney (Fig. 5). In fish treated with 4.0 mg Ga L^{-1}, degenerative changes of tubular epithelial cells and ectasis of Bowman's capsules were observed (Fig. 6). Severe renal lesions were also found in fish exposed to the highest concentration (8.0 mg Ga L^{-1}). Similarly, damage to tubular epithelial cells and degeneration of glomeruli became more prominent (Fig. 7).

In addition to the reabsorption of salt and glucose, the renal tubular epithelium of fish plays a major role in the excretion of pollutants like heavy metals. Thus heavy metals such as mercury or cadmium are highly likely to have a chronic effect on these cells (Ferguson 1989). Our results indicate that gallium-induced histopathological changes observed in the kidney of juvenile carp are similar to pathological changes observed in the same species due to other heavy metal toxicities (Singhal and Jain 1997). Gallium has been shown to produce nephrotoxic damage in mammalian systems. Renal tubular damage can cause uncompensated acidosis, decreased serum Na^+ and K^+ levels, and increased sugar as well as urea nitrogen in the blood (Venugopal and Luckey 1978). More studies such as biochemical and accumulation analyses of the kidney are required to follow-up on the present study in order to understand the effects on common carp.

Frequent use of gallium compounds in semiconductor manufacturing has been accompanied by increasing amounts of toxic materials released as potential toxic wastes, which are harmful to health and the environment (Chelton et al. 1991). Heavy metals (including arsenic) are some of the most-active polluting substances as they can cause serious impairment to metabolic, physiological, and even structural systems when high concentrations are present in the environment. Although heavy metals are often referred to as a common group of pollutants, each metal produces different problems in freshwater environments, and therefore metals have to be considered separately and in combination as well (Lloyd 1992). Because many wastewater discharges contain a mixture of pollutants, the combined effect of gallium with copper or zinc has to be carried out. Retardation in body growth by gallium and injuries to the gills and kidney are clearly evident from the present study. Gallium has served as a tool to localize the lesion sites of toxic action. In mammals, gallium acts as a neuromuscular poison and causes renal damage. Photophobia, blindness, and paralysis have been reported in rats. Large doses given to rats caused renal precipitates consisting of gallium, calcium, and phosphate (Newman et al. 1979). All of these findings support gallium being a potential pollutant, although no adverse effects following industrial exposure have been reported to date.

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