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Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology

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Abstract

Gallium (Ga) is one of the intermetallic elements that are increasingly being used in making high-speed semiconductors such as gallium arsenide. The purposes of this study were to investigate the effects of gallium on acute toxicity, on serum biochemical variables as well as on erythrocyte morphological changes in the blood stream of common carp (*Cyprinus carpio*). Median lethal concentrations were determined in acute tests. The 96-h LC_{50} value was 19.78 (18.49–21.16) $mg\,l^{-1}$. Common carp were exposed to different gallium concentrations (2.0, 4.0, and 8.0 $mg\,l^{-1}$) for 28 days in laboratory toxicity tests. Means of the measured serum biochemistry parameters (including glucose, blood urea nitrogen, creatinine, cholesterol, and triglyceride) of these exposed groups significantly differed from those of the untreated group. Deformation of erythrocytes suggest disturbance of respiration as an additional indicator of Ga exposure. Our results suggest that 2.0 $mg\,l^{-1}$ is proposed as a biologically safe concentration which can be used for establishing tentative water quality criteria concerning of same size common carp. In addition, serum biochemical parameters as well as erythrocyte morphological changes are promising clinical diagnostic tools for assessing the effects of gallium compounds on common carp.

Keywords: Gallium; Common carp; LC_{50}; Serum chemistry; Erythrocyte

1. Introduction

Compound semiconductors, such as GaAs, GaP, and AlGaAs, are important materials in the manufacture of integrated circuits and optoelectronic devices in the semiconductor industry (Venugopal and Luckey, 1978; Robinson, 1983). 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. Bustamante et al. (1997) indicated that the semiconductor element arsenic (0.01 μM) is able to induce apoptosis in rat thymocytes and highter doses of arsenic (10 μM) induced cell death by necrosis. Lin and Hwang (1998) showed that the 96-h LC_{50} of gallium for tilapia larvae (*Oreochromis mossambicus*) was estimated to be 204 μM. Furthermore, aqueous waste streams can contain from 200 to 400 $mg\,l^{-1}$ of each dissolved metal in the wet polishing process of gallium arsenide (Sturgill et al., 2000). Research on gallium compounds for use 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; Sturgill et al., 1999).

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Gallium can interfere with calcium uptake; the element is a potent inhibitor of protein synthesis and the heme pathway enzyme aminolevulinic acid dehydratase (Hoyes et al., 1992). Gallium also appears to inhibit DNA synthesis by its action on ribonucleotide reductase (Riaz et al., 1995). Previous reports indicated that gallium compounds might cause bone marrow depression, testicular toxicity, and hemorrhagic nephritis in mammals (Webb et al., 1987; Aoki et al., 1990; Omura et al., 1996). In teleosts, tilapia larvae (*O. mossambicus*) show retardation in body growth with sublethal levels of gallium (Lin and Hwang, 1998). However, there is limited knowledge of the adverse effects of gallium on aquatic animals.

Industrial spills can lead to high concentrations of toxic materials in rivers, affecting freshwater ecosystems with acute and chronic toxicity. Fish are particularly sensitive to environmental contamination of water. Therefore, pollutants may significantly damage certain physiological and biochemical processes when they enter the organs of fishes (Teh et al., 1997). In order to better understand the impacts of gallium on freshwater systems, selected studies on this metal need to be conducted.

Measurement of serum biochemical parameters can be especially useful to help identify target organs of toxicity as well as the general health status of animals, and is advocated to provide early warning of potentially damaging changes in stressed organism (Folmar, 1993; Jacobson-Kram and Keller, 2001). Total serum protein (TP), the majority of serum proteins which are synthesized in the liver, is used as an indicator of liver impairment. Decreased concentrations of TP are common in many disease states and may result from impaired synthesis (liver disease), reduced absorption, or protein loss (Bernet et al., 2001). Changes in glucose (GLU) concentrations are most often associated with renal injury. Serum concentrations of glucose are regulated by complex interactions of hormones such as glucagon and cortisol. However, environmental stress can also cause marked elevations in plasma glucose levels as well (Martin and Black, 1998). Further, nutritional status can have a significant influence on glucose level. Decreased serum glucose concentrations can result from liver failure and being undernourished. Blood urea nitrogen (BUN), is a major nitrogen-containing metabolic product of protein catabolism. In mammals, increased concentrations of urea occur due to a high-protein diet or to renal lesions (Jacobson-Kram and Keller, 2001). Creatinine (CR) can provide similar information to that of blood urea nitrogen in renal disease or postrenal obstruction or leakage. Whereas increased concentrations may reflect kidney dysfunction due to structural damage, low concentrations have no clinical significance (Bernet et al., 2001; Jacobson-Kram and Keller, 2001). Cholesterol (CHOL) is an essential structural component of cell membranes, the outer layer of plasma lipoproteins, and the precursor of all steroid hormones. The primary function of triglyceride (TG) is to store and provide cellular energy, and like serum protein and cholesterol levels, can be used as an indicator of nutritional status.

Erythrocytes are the most abundant cell type found in the peripheral blood and function in respiration by transporting oxygen to and carbon dioxide from body tissues (Michael and Stoskope, 1992). Many studies have indicated that heavy metal ions, such as copper, cadmium, and mercury ions, induce lysis of mammalian erythrocytes and may cause the accelerated destruction of erythrocytes (Arbuthnott, 1962; Adams et al., 1979; Ribarov and Benov, 1981; Ichikawa et al., 1987; Kotsanis et al., 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. The purpose of this study was to investigate the effect of sublethal gallium concentrations on biochemical parameters and erythrocyte morphology in carp.

2. Materials and methods

Common carp were obtained from the Chupei Branch of the Taiwan Fisheries Research Institute. Fish were transported to a glass aquarium 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 $mg\,CaCO_3\,l^{-1}$, ammonia < 0.5 $mg\,l^{-1}$, and nitrite 0.05–0.1 $mg\,l^{-1}$) was used. Fish were acclimated for 14 days and fed an aquarium fish mixture every 2 days. The temperature was maintained at 25.0 ± 0.5 °C, and the photoperiod was set at 12 h of light and 12 h of dark during the entire experiment. Common carp (12 weeks old, 2.3 ± 0.19 g in body weight) were used for acute and chronic tests 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 common 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).

Common carp for 28-day chronic tests were randomly placed in triplicate 100-l glass aquaria. Every aquarium contained four fish which were exposed to test solutions of the following concentrations: 0.0, 2.0, 4.0, and 8.0 mg l^{-1}, respectively. Six fish per exposure concentration were anesthetized with MS-222 (Sigma Chemical, St. Louis, MO) after 28 days of exposure. Blood samples were taken from each fish by puncture of the caudal vessel.

After blood removal, blood smears were made immediately. Smears made from blood samples were air dried for 1 h and then fixed in 95% methanol at 4 °C (Michael and Stoskope, 1992). Slides were stained with a modified Wright stain (Sigma Chemical, St. Louis, MO), and a cover slip was placed on top using glycerol.

In the serum biochemical analysis, blood samples were prepared using the method described by Bernet et al. (2001) with slight modification. Blood was allowed to coagulate at room temperature for 2 h. Serum was obtained by centrifugation of an amount of blood at 1500 × g (for 10 min at 4 °C) and than stored at −80 °C for several weeks until analysis. The concentration of total protein (TP), glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), cholesterol (CHOL), and triglyceride (TG) were measured by using a Johnson & Johnson Ektachem 250 biochemical analyzer. Test kits from Johnson & Johnson were used for determinations (New York, USA).

All values of the enzyme assay were analyzed statistically by analysis of variance (ANOVA) using SAS statistical software (SAS, 1988). Duncan's multiple range test was used to evaluate the mean difference among individual groups at the 0.05 significance level.

3. Results

According to the static renewal method for acute toxicity testing (Buikema et al., 1982), median lethal concentrations (LC_{50}) of gallium for common carp were obtained. Values for the 48-, 72-, and 96-h LC_{50} is 28.81, 22.11, and 19.78 mg l^{-1}, respectively. Toxicity increased with increasing concentration (Table 1).

Sublethal levels of gallium were equivalent to approximately 10%, 20%, and 40% of the 96-h LC_{50} value (19.78 mg l^{-1}) for 28-day toxicity testing. Therefore no mortality was recorded during the whole experiment period for all exposure concentrations studied. With the exception of TP, all serum chemical parameters in exposed groups were found to significantly differ between exposed groups and the untreated group after a 28-day exposure time (Table 2). BUN, CR, CHOL, and TG concentrations at higher exposure levels (4.0 and 8.0 mg Ga l^{-1}) exhibited higher values than those of the control groups; values recorded were 50–100% higher than those of the control group. In contrast, GLU concentrations in serum of treated carp (4.0 and 8.0 mg Ga l^{-1}) were significantly lower than those of the control groups after a 28-day exposure.

Normal erythrocytes of carp have an oval shape with a rounded to oval central nucleus with densely packed chromatin (Fig. 1). The present study shows erythrocyte morphological alterations in carp exposed to gallium (Figs. 2 and 3). A high percentage of red blood cells were in the process of losing their normal outline and cytoplasm according to the peripheral blood smear examination at higher exposure levels (4.0 and 8.0 mg Ga l^{-1}).

4. Discussion

Heavy metals (including arsenic) are some of the most-active polluting substances as they can cause serious impairment to circulatory, metabolic, physiological,

Table 1
Median lethal concentrations (LC_{50}, mg Ga l^{-1}) of gallium to common carp (*Cyprinus carpio*)

48 h	72 h	96 h
28.81 (21.92–37.88)	22.11 (20.69–23.63)	19.78 (18.49–21.16)

The 95% confidence limits are given in parentheses.

Table 2
Serum biochemical parameters of common carp (*C. carpio*) exposed to gallium

	Control	2.0 mg Ga l^{-1}	4.0 mg Ga l^{-1}	8.0 mg Ga l^{-1}
Total protein (g dl^{-1})	3.13 ± 0.24^{a}	2.97 ± 0.18^{a}	3.10 ± 0.27^{a}	3.09 ± 0.18^{a}
Glucose (mg dl^{-1})	78.67 ± 3.20^{a}	76.18 ± 6.47^{a}	58.77 ± 4.99^{b}	56.52 ± 12.78^{b}
Blood urea nitrogen (mg dl^{-1})	0.97 ± 0.56^{a}	1.13 ± 0.65^{a}	2.00 ± 0.22^{b}	2.00 ± 0.64^{b}
Creatinine (mg dl^{-1})	0.47 ± 0.05^{a}	0.47 ± 0.05^{a}	0.79 ± 0.09^{b}	0.93 ± 0.10^{c}
Cholesterol (mg dl^{-1})	136.67 ± 10.41^{a}	172.00 ± 10.12^{b}	198.96 ± 21.75^{b}	202.35 ± 22.12^{b}
Triglyceride (mg dl^{-1})	128.33 ± 7.63^{a}	134.61 ± 10.78^{a}	166.67 ± 31.24^{b}	210.33 ± 16.84^{c}

All values are given as the mean ± SD; $n = 6$. Values in the same row with different superscripts differ at $p < 0.05$.

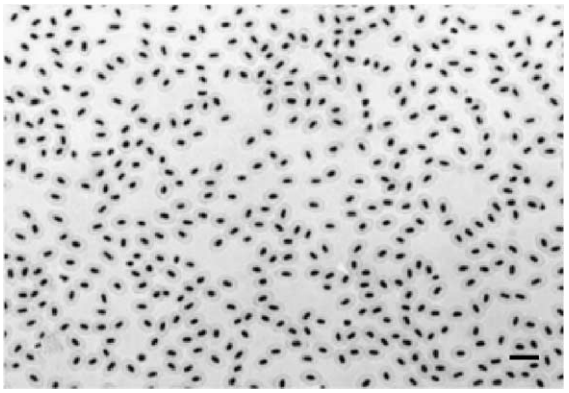

Fig. 1. Blood smear of common carp exposed to Ga-free water for 28 days (modified Wright stain, bar = 15 μm).

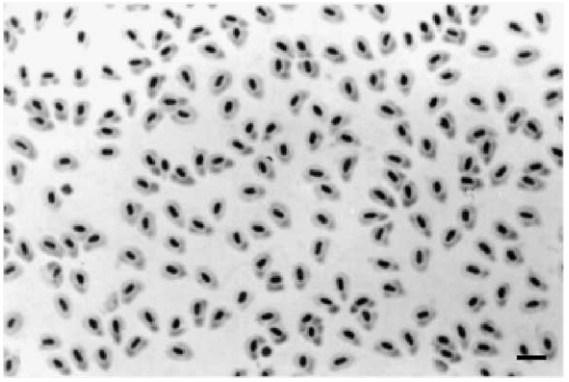

Fig. 2. Blood smear of common carp exposed to 4.0 mg Ga l^{-1} for 28 days (modified Wright stain, bar = 15 μm).

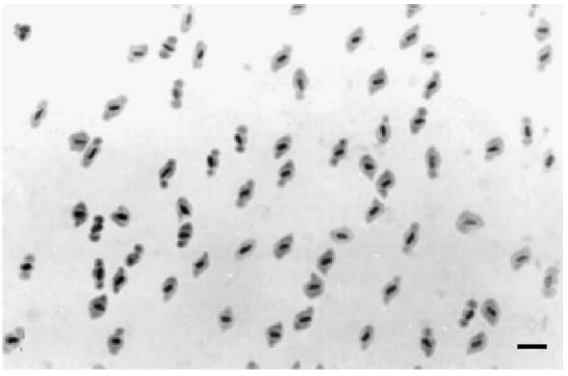

Fig. 3. Blood smear of common carp exposed to 8.0 mg Ga l^{-1} for 28 days (modified Wright stain, bar = 15 μm).

and even structural systems when high concentrations are present in aquatic ecosystems (Shugart et al., 1992). Although heavy metals are often referred to as a common group of pollutants, individual metals pose different problems in freshwater environments, and therefore they have to be considered separately (Lloyd, 1992).

Much more extensive biochemical toxicological research has been conducted in mammals than in fish. However, it is not surprising that many biochemical similarities exist among vertebrate species (Hochachka and Mommsen, 1995). The kidney and liver have been proposed as the major target organs for environmental contaminants such as heavy metals, and they are important organs for metabolic waste excretion and heavy metal elimination in fish (Laurent and Dunel, 1980). The increase in BUN concentration in serum has frequently been used in fish as an indicator of gill and kidney dysfunction (Bernet et al., 2001). In addition to BUN, CR concentrations in serum of intoxicated carp were significantly higher than those of control carp after 28 days of gallium exposure. CHOL and TG have been used for demonstrating the nutritional status in animals. Increased serum cholesterol concentrations can result from damage of liver or nephrotic syndrome (Yamawaki et al., 1986; Seyit et al., 2000). TG is used to evaluate lipid metabolism; high concentrations may occur with nephritic syndrome or glycogen storage disease (Bernet et al., 2001). The data, which show that serum GLU concentrations tend to decline at a faster rate in treated than in control fish, suggest that gallium-treated fish are in an undernourished state or are experiencing liver failure (Jacobson-Kram and Keller, 2001).

Morphological alterations in erythrocytes suggest obstruction of gas exchange as an additional process of Ga exposure. Although the affinity of heavy metals for SH-groups in membrane proteins can affect membrane conformation and permeability (Pentreath, 1976), Ribarov and Benov (1981) suggested that the peroxidation of membrane lipids is also a possible mechanism of damage to erythrocyte membranes treated with metals, and thus fostered understanding of the underlying toxicological mechanism in future studies.

Comparing the toxicity of gallium with 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 the zinc. The 96-h LC_{50} value of gallium for 3-day-old tilapia larvae (*O. mossambicus*) was estimated to be 14.32 mg l^{-1} (Lin and Hwang, 1998), indicating that tilapia is might be more tolerant to gallium exposure than common carp. Further, almost no toxic effect was seen at 2.0 mg Ga l^{-1} which is equivalent to 10% 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). Thus, 2.0 mg l^{-1} is proposed as a biologically safe concentration which can be used for establishing tentative water quality criteria concerning of same size common carp.

5. Conclusion

Because of the widespread, large-volume, high-frequency use of gallium compounds in semiconductor manufacturing, we must be aware of their toxicity in natural ecosystems. 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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