

## Effects of Transition Metal Gallium on the Serum Biochemistry and Erythrocyte Morphology of Goldfish (*Carassius auratus*)

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Heavy metals such as gallium (Ga) cause serious physiological damage to exposed organisms, mostly of aquatic species. Ga one of the inter-metallic, transition elements increasingly being used in making high-speed semiconductors, such as Ga arsenide. The purposes of this study were to investigate the effects of Ga on acute toxicity, serum biochemical changes, and erythrocyte morphological changes in the blood stream of goldfish (*Carassius auratus*). Median lethal concentrations were determined in acute tests. The 96 hr LC<sub>50</sub> value was 9.15 mg/ml. Goldfish were exposed to different Ga concentrations (2.0, 4.0, and 8.0 mg/ml) for 30 days to assess its toxic effects. The results indicate that the measured serum biochemistry parameters (including glucose, blood urea nitrogen, creatinine, cholesterol, and triglyceride) of the Ga-exposed fish groups differed significantly from the untreated fish group. In addition, a change in the erythrocytes' morphology at a high concentration (8.0 mg/ml) of Ga exposure shows respiratory problems. Our results suggest that 2.0 mg/ml is proposed as a biologically safe concentration that can be used for establishing tentative water quality criteria concerning the same-size goldfish.

**Key words** : Erythrocyte, gallium, goldfish, LC<sub>50</sub>, serum biochemistry

### Introduction

Compound semiconductors, such as gallium arsenide, gallium phosphide and aluminium gallium arsenide, are important materials in the manufacture of integrated circuits and optoelectronic devices in the semiconductor industry [24, 25]. Manufacturing processes devoted to the fabrication of gallium based semiconductor devices generate large volumes of wastes that contain the toxic metal arsenic as well as gallium. Bustamante *et al.* [3] indicated that the semiconductor element arsenic (0.01 M) is able to induce apoptosis in rat thymocytes and higher doses of arsenic (10 M) induced cell death by necrosis. Lin and Hwang [13] showed that the 96 hr LC<sub>50</sub> of gallium for tilapia larvae (*Oreochromis mossambicus*) was estimated to be 204 µM. Furthermore, aqueous waste streams can contain from 200 to 400 mg/ml

of dissolved metal in the wet polishing process of gallium arsenide [23].

Gallium was reported to interfere with calcium uptake of cell and in turn inhibit cellular function such as protein synthesis and related pathways [7]. Gallium also appears to inhibit DNA synthesis by its action on ribonucleotide reductase [18]. Previous reports indicated that gallium compounds might cause bone marrow depression, testicular toxicity, and hemorrhagic nephritis in mammals [9, 16]. In teleosts, tilapia larvae (*O. mossambicus*) show retardation in body growth with sublethal levels of gallium [13].

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 [4].

In order to understand the impacts of gallium on freshwater systems, selected studies on this metal need to be conducted in a potent biomarker of environmental contaminants like goldfish [14]. Measurement of serum biochemical pa-

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parameters such as total protein, total cholesterol, creatinine etc were useful to identify toxicity level in target organs as well as the general health status of animals, and is advocated to provide early warning of potentially damaging changes in stressed organism [1, 9].

Moreover, several studies have indicated that heavy metal ions, such as copper, cadmium, and mercury ions, induce lysis of mammalian erythrocytes and accelerated its destruction [8, 10, 19] as they are the most abundant cell type found in peripheral blood and function as transporter of O<sub>2</sub> and CO<sub>2</sub> to and from the body tissue respectively [15]. As the goldfish is an important freshwater ornamental fish species which could be easily cultured and used 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 goldfish.

## Materials and Methods

### Fish

Healthy goldfish were obtained from the Fisheries Research Institute, Pyeosen, Jeju-do, South Korea. 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/ml, hardness CaCO<sub>3</sub> 38~45 mg/ml, ammonia < 0.5 mg/ml, and nitrite 0.05~0.1 mg/ml) was used. Fish were acclimatized for 14 days and fed with commercial fish feed (TetraBits<sup>®</sup> complete, Germany) twice a day. The temperature was maintained at 25.0±0.5°C, and the photoperiod was set at 12 hr of light and 12 hr of dark during the entire experiment. Goldfish (4 weeks old, 1.3±0.25g in body weight) were used for acute and chronic tests in the initial experiments. These experimental procedures are in accordance with the ethical committee guidances of Jeju National University (2016-0011).

### Lethal Concentration 50

Gallium sulfate (purity 99.999%) was purchased from Sigma (Sigma-aldrich., USA). A stock solution was prepared to 1,000 mg/ml gallium in 0.1% nitric acid. Laboratory static renewal tests were conducted to determine the median lethal concentration (LC<sub>50</sub>) for goldfish. Ten fish of similar size were randomly sampled and placed in glass tank. After 24 hr 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/ml) for 96 hr 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 hr. The LC<sub>50</sub> of gallium and its 95% confidence limits for goldfish were calculated [26].

### Expose of gallium

About 480 goldfish were randomly grouped into four groups in 100 fish glass aquaria in triplicate. Each group contained forty fish which were exposed to following concentrations of gallium: 0.0, 2.0, 4.0, and 8.0 mg/ml. Ten fish from each group per exposure concentration were anesthetized with MS-222 (Sigma Chemical, St. Louis, MO) after 30 days of exposure.

### Erythrocyte morphology

Blood samples were taken from each fish by puncture of the caudal vessel and blood smears were made immediately; air dried for 1 hr and then fixed in 95% methanol at 4°C [15]. Slides were stained with a modified Wright stain (Sigma Chemical, USA), and a cover slip was mounted using glycerol.

### Serum biochemistry

For serum biochemical analysis, blood samples were prepared using the method described by Bernet *et al.* [1] with little modification. Blood was allowed to coagulate at room temperature for 2 hr. Serum was obtained by centrifugation of an amount of blood at 1,500 g (for 10 min at 4°C) and then stored at -80°C for several weeks until analysis. The concentration of glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), cholesterol (CHOL), and triglyceride (TG) were measured by using a CH100 Plus, Daekwang Meditech, Korea. All values of the enzyme assay were analyzed statistically by analysis of variance (ANOVA) using SPSS statistical software [22]. Duncan's multiple range tests was used to evaluate the mean difference among individual groups ( $p \leq 0.005$ ).

## Results

### Lethal Concentration 50

According to the static renewal method for acute toxicity testing [2], median lethal concentrations (LC<sub>50</sub>) of gallium for goldfish were obtained. Values for the 48, 72, and 96 hr LC<sub>50</sub> is 23.07±0.23, 16.14±0.11 and 9.15±0.21 mg/ml,

Table 1. Median lethal concentrations (LC<sub>50</sub>, mg/ml) of gallium to goldfish (*Carassius auratus*)

48 hr	72 hr	96 hr
23.0±0.23	16.14±0.11	09.15±0.21*

All values are given as the mean ± SD; n = 10. Values with \*differ significantly at  $p \leq 0.005$ .

respectively. Toxicity increased with increasing concentration (Table 1). Sublethal levels of gallium were used in 30 days chronic toxicity experiments. Hence, no mortality was recorded during the whole experiment period for all four concentrations studied.

### Serum biochemistry

All serum biochemical parameters of gallium-treated fish groups differ significantly ( $p \leq 0.005$ ) from the untreated control fish group after 30 days of exposure time (Table 2). BUN, CR, CHOL, and TG concentrations at higher exposure levels (4.0 and 8.0 mg/ml) 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 goldfish treated (4.0 and 8.0 mg/ml) were significantly lower than those of the control groups after 30 days exposure.

### Erythrocyte morphology

Normal erythrocytes of control fish have an oval shape with densely packed nucleus (Fig. 1). However, gallium exposed fish shows round shaped erythrocyte. (Fig. 2). A high percentage of red blood cells were in the process of losing their normal outline and nuclear material which clear from the peripheral blood smear examination at higher exposure levels (8.0 mg/ml).

## Discussion

Heavy metals are the most-active polluting substances as

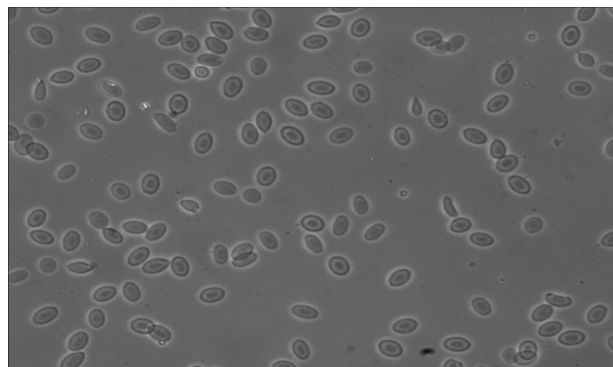


Fig. 1. Blood smear of control goldfish.

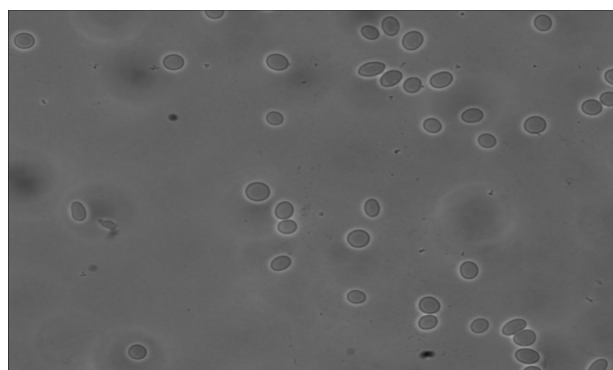


Fig. 2. Blood smear of goldfish exposed to gallium 8.0 mg/ml for 30 days.

they can cause serious circulatory, metabolic, and physiological impairment on the exposed organism. 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 [14]. 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 [18].

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

Table 2. Serum biochemical parameters of goldfish (*Carassius auratus*) exposed to gallium

Parameter	Control	2.0 mg/ml	4.0 mg/ml	8.0 mg/ml
Total protein (g/dl)	2.26±0.24 <sup>a</sup>	1.03±0.12 <sup>a</sup>	2.43±0.21 <sup>a</sup>	3.11±0.23 <sup>a</sup>
Glucose (mg/dl)	64.11±2.10 <sup>a</sup>	59.28±3.16 <sup>a</sup>	53.17±2.77 <sup>b</sup>	50.61±9.67 <sup>b</sup>
Blood urea nitrogen (mg/dl)	0.74±0.56 <sup>a</sup>	0.93±0.34 <sup>a</sup>	1.05±0.3 <sup>b</sup>	1.25±0.43 <sup>b</sup>
Creatinine (mg/dl)	0.32±0.05 <sup>a</sup>	0.33±0.15 <sup>a</sup>	0.47±0.06 <sup>b</sup>	0.61±0.2 <sup>c</sup>
Cholesterol (mg/dl)	121.3±11.02 <sup>a</sup>	153.5±09.23 <sup>b</sup>	169.5±15.33 <sup>b</sup>	182.4±20.2 <sup>b</sup>
Triglyceride (mg/dl)	104.5±3.5 <sup>a</sup>	115.15±9.4 <sup>a</sup>	120.26±7.1 <sup>b</sup>	135.25±8.15 <sup>c</sup>

All values are given as the mean ± SD; n = 10. Values in the same row with different superscripts differ at  $p \leq 0.005$ .

excretion and heavy metal elimination in fish [12].

The increase in BUN concentration in serum has frequently been used in fish as an indicator of gill and kidney dysfunction [1]. In addition to BUN, CR concentrations in serum of intoxicated goldfish were significantly higher than those of control fish after 30 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 [21]. TG is used to evaluate lipid metabolism; high concentrations may occur with nephritic syndrome or glycogen storage disease [1]. The data, which show that serum GLU concentrations tend to decline at a faster rate in treated than in control goldfish, suggest that gallium-treated goldfish are in an undernourished state or are experiencing liver failure [9].

Morphological alterations in goldfish erythrocytes suggest obstruction of gaseous 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 [17], previous study had suggested that the peroxidation of membrane lipids is also a possible mechanism of damage to erythrocyte membranes treated with metals [19], and thus encouraging further studies to know the exact toxicological mechanism in future.

Comparing the toxicity of gallium with zinc (96 hr LC<sub>50</sub>: 17 mg/ml) for the carp species [5, 11], it is clear that the toxicity of gallium is no stronger than that of the zinc. The 96 hr LC<sub>50</sub> value of gallium for 3 days old tilapia larvae (*O. mossambicus*) was estimated to be 14.32 mg/ml [13], indicating that tilapia is might be more tolerant to gallium exposure than goldfish. Further, almost no toxic effect was seen at 2.0 mg/ml which is equivalent to 10% of the 96 hr LC<sub>50</sub> value, and is in good agreement with the concept of a safe level (one-tenth of the 96 hr LC<sub>50</sub> value) as described by Sprague [17]. Thus, 2.0 mg/ml is proposed as a biologically safe concentration which can be used for establishing tentative water quality criteria concerning of same size goldfish.

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### 초록 : 전이금속 갈륨이 금붕어(*Carassius auratus*)의 적혈구 및 혈청의 생화학반응에 미치는 영향

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갈륨은 갈륨비소와 같은 고속 반도체 제작에 사용되는 금속간 화합물 중 하나이다. 본 연구의 목적은 금붕어 (*Carassius auratus*)에 갈륨을 적용 시 일어나는 혈청의 생화학 변수, 급성독성 및 적혈구 형태의 변화에 대해 알아 보고자 한다. LC<sub>50</sub>은 96시간 쯤 갈륨 농도 9.15 mg/ml로 나타났다. 갈륨을 농도별(2.0, 4.0, 8.0 mg/ml)로 금붕어에 노출시켜 28일 동안 독성실험을 하였다. 독성실험 결과 혈청의 생화학(글루코즈, 혈액요소질소, 크레아티닌, 콜레스테롤 및 중성지방)반응에서 갈륨 미처리 그룹과 다른 결과가 나타났다. 갈륨의 노출에 따른 적혈구의 변형으로 인한 호흡장애를 유발하는 것으로 사료된다. 실험에 사용된 금붕어와의 동일한 크기에 갈륨을 적용할 때 생물학 적으로 안전한 농도는 2.0 mg/ml로 사료된다.