ISSN : 0974 - 7451 Volume 9 Issue 3

Environmental Science
An Indian Journal
Current Research Paper

ESAIJ, 9(3), 2014 [113-117]

Oxygen consumption and histochemical studies of common carp (*Cyprinus carpio*) exposed to various levels of gallium

Jen-Lee Yang[1]*, Tung-Jer Hu[2]

[1]Department of Life Science, Chinese Culture University, 55, Hwa-Gung Road, Yangmingshan, Taipei, 111, (TAIWAN)

[2]Department of Computer Application Engineering, Lanyang Institute of Technology No.79, Fushin Rd., Touchen Town, Yilan County, 261, (TAIWAN)

E-mail : d88225003@yahoo.com.tw

ABSTRACT

Gallium is one of the intermetallic elements which are increasingly being used for the manufacture of semiconductor devices. The purposes of this study were to investigate its oxygen consumption and liver histochemistry on common carp (*Cyprinus carpio*). Common carp were exposed to different levels of antimony (1.0, 2.0, 4.0, and 8.0 mg/L) over a 28-day test period and a 14-day recovery period. After 30 min (acute), there was an increase in the amount of oxygen consumed in all exposed groups. On days 14 and 28 decreases in oxygen consumption were significant ($p < 0.05$) for the higher-exposure level groups (4.0 and 8.0 mg/L). The increased nuclei shrinkage and cellular fragmentation of the liver were found in common carp after using terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling. Changes in oxygen consumption and histopathological performances could be considered as promising clinical diagnostic tools for assessing the toxic effects of gallium compounds on common carp.

KEYWORDS

Gallium;
Toxicity,
Oxygen consumption;
Hepatocyte;
TUNEL.

INTRODUCTION

Gallium compounds such as GaAs and AlGaAs are important materials for the manufacture of integrated circuits and optoelectronic devices in the semiconductor industry[6]. 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. Discharged wastewater from the gallium arsenide wet polishing process can contain from 200 to 400 mg L^{-1} of each dissolved metal. However, gallium is not listed as a hazardous waste under local regulations (in Taiwan), but is listed as hazardous in California in the US[5].

Gallium can interfere with calcium uptake; the element is a potent inhibitor of protein synthesis and the heme pathway enzyme aminolevulinic acid dehydratase[10]. Gallium also appears to inhibit DNA synthesis by its action on ribonucleotide reductase[14]. Previous reports indicated that gallium compounds might cause bone marrow depression, testicular toxicity, and hemorrhagic nephritis in mammals[4,12,18]. In teleosts, Yang and Chen[8] indicated that the 96-h LC_{50} of gallium for common carp larvae was estimated to be 12.55

mg/L, and showed retardation in body growth with sublethal concentrations of gallium.

Many studies examining of oxygen consumption has been widely considered as a critical factor to evaluate the physiological response and a useful variable for early warning monitor of aquatic organisms (Chinni et al. 2000). Like most fish, common carp are oxygen regulators, they maintain their oxygen consumption at a constant level along a gradient of environmental oxygen concentrations, until a critical oxygen concentration is reached, and below which oxygen consumption begins to fall. Under conditions of stress, this critical oxygen concentration is likely to increase, reflecting the decreased capacity of the fish to cope with environmental perturbation. For common carp and trout, a shift in critical oxygen concentration to higher oxygen concentration has been observed when exposed to low pH or other stress[9]. And metabolic response to changes in oxygen availability may vary, depending on the physiological state of the animal, level of activity and temperature[17].

The liver is an important organ involved in metabolic processes and in detoxification of xenobiotics. In some situations, poisonous materials accumulate in the liver to toxic levels and cause pathological alterations[1]. The type of liver injury is often dependent upon not only the particular agent and its mechanism of action but also on the length of exposure[3]. The prolonged hepatic ultrastructural effects of gallium need to be better understood.

Common carp (*Cyprinus carpio*) is an important cultured fish species in fishponds near semiconductor manufacturing districts in Taiwan, it is a potential species to study the toxicity of semiconductor-related metals. The purposes of this study were to investigate the effects of sublethal gallium concentrations on oxygen consumption and liver histochemical changes in the common carp, so that these evidences can then be used to evaluate the possible adverse effects of gallium. And provide essential information which can be used to objectively institute measures to minimize pollution by gallium and its impacts on aquatic environment. Meanwhile, we examined the liver injury evoked by gallium in common carp and explored the possibility that related research may also apply to aquatic animal modeling in the future.

MATERIALS AND METHODS

Animal

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; hardness 38-45 mg $CaCO_3$/L) was used during the entire experiment. The temperature was maintained at 25.0 ±0.5 ºC, 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 oxygen consumption measurements; juvenile (12 weeks old, 2.3 ± 0.19 g in body weight) were used for histochemical studies 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 gallium in 0.1% nitric acid).

Oxygen consumption analysis were prepared using the method described by Chinni et al.[13] with slight modification, groups of 20 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, 4.0, and 8.0 mg/L, respectively in triplicate. Twice a week 50% of the water was renewed with standard water containing gallium to maintain the environmental condition in the entire experiment period. Exposure time was 4 weeks and 2-weeks recovery period in Ga-free water.

Both control and exposed samples were taken at intervals of acute (after 30 min), day-14, day-28, and day-42 for estimation of oxygen consumption. Oxygen consumption tests are customarily measured by merely sealing fish in a respiratory jar of 325 ml capacity with oxygen electrode (Microprocessor Oximeter, WTW, Germany). Kept all respiratory jars contain up to 7 mg O_2/L before initial measuring. At each interval, 2 fry were put into the respiratory jar with an acclimatization of 30 mins as recorded earlier, and then oxygen consumption was estimated. Allowing them to deplete the oxygen until death occurs, and the residual dissolved oxygen is measured by multiple range temperature and oxygen analyzer and recorder (Yokogawa, Japan).

Oxygen consumption (QO_2, mg/O_2/kg/h) were calculated as follow:

$$QO_2 = \Delta ppm \times 1/BW \times V \times 1/t$$

where QO_2 is the amount of oxygen (Δppm) consumed in the interval t (h). BW is the wet body weights (Kg) at the start and at the end of that testing period.

Light microscopic in situ nick end labeling

Juvenile common carp for histochemical studies were randomly placed in 50-L glass aquarium. Every aquarium contained 5 fish. Liver of each fish was examined at 12, 24 and 36 hr after intraperitoneal injection of 40 mg/Kg gallium solution. Control fish were injected with same volume of physiological saline. Three fish per group were anesthetized with MS-222 (Sigma Chemical, MO, U.S.A) and sacrificed. Liver was removed for TUNEL assay.

Small pieces of carp liver from control and treated specimens were collected, fixed in 4% buffered formalin, and processed routinely for examination using paraffin section. DNA fragments were detected *in situ* on paraffin sections by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) method (Gavrieli et al. 1992) was employed for liver sections with slight modification using an *in situ* detection kit (Oncor, Gaithersberg, Germany). Sections (6 μm) were deparaffinized, rinsed with PBS, treated with proteinase K (20 μg ML^{-1}) to strip the nuclear proteins, and then quenched in endogenous peroxidases with 2% H_2O_2 in PBS. DNA 3'OH-ends were labeled with digoxigenin-conjugated dUTP. Bound digoxigenin was detected by incubation with peroxidase-conjugated antibody against digoxigenin at 37!for 2 hr and then developed with 50 mM Tri-HCl (pH 7.4)-0.1% DAB-0.02% H_2O_2. After TUNEL staining, counterstain was done with 0.1 M sodium acetate-2% methylgreen (pH 4.0). Substituting the TdT enzyme solution with distilled water in the reaction step carried out a negative control of the reaction.

Statistical analysis

All values of oxygen consumption measurements were analyzed statistically by analysis of variance 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.

RESULTS

Oxygen consumption

The results on rates oxygen consumption for control and exposed common carp were presented in Figure 1. After 30 min, there was an increase in amount oxygen consumption in exposed common carp, a maximum increase of 57.5% was observed at the highest exposure concentration (8.0 mg/L), the increase was significant ($p < 0.05$) in exposed common carp in relation to gallium concentrations. On day-14, day-28, and day-42 (recovery period), decrease in oxygen consumption was significant ($p < 0.05$) at higher exposure level groups (4.0 and 8.0 mg/L).

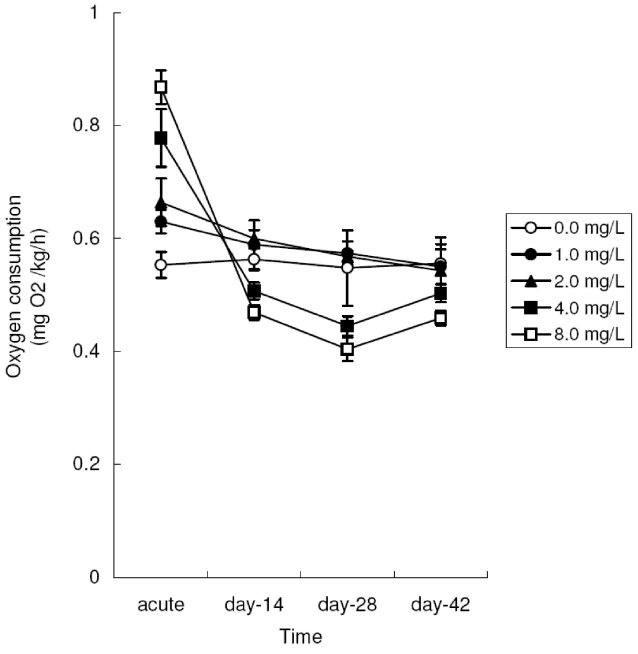

Figure 1 : Effect of gallium on the oxygen consumption (mg O_2/kg/h) of fry common carp during the experimental period (mean ± SD, *n* = 3).

In addition, the percent decrease in oxygen consumption over their respective controls from acute (after 30 min) to day-42 at lower exposure levels (1.0 and 2.0 mg/L). However, the increase in oxygen consumption observed in recovery period (day-42) over their respective same level groups on day-28 at higher exposure levels (4.0 and 8.0 mg/L).

Light microscopic observation

In situ nick end labeling study using light microscopy showed no positive staining in the control and 12

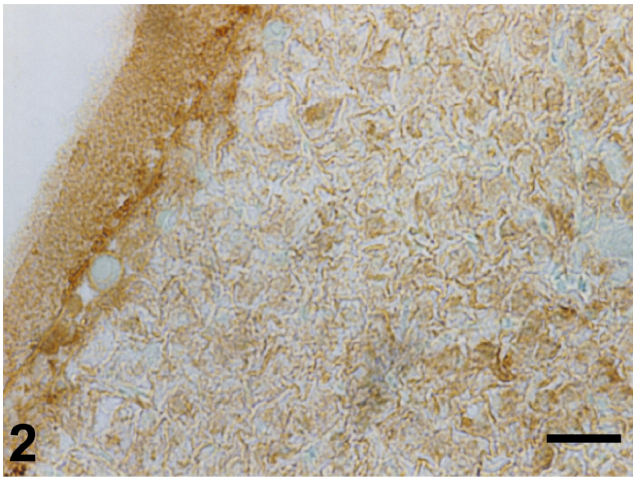

Figure 2 : TUNEL assay of junvile common carp (*Cyprinus carpio*) exposed to gallium. The liver tissue showed weak staining in the untreatment and 12 hr after intraperitoneal injection of 40 mg/L gallium. (bar = 0.15 mm)

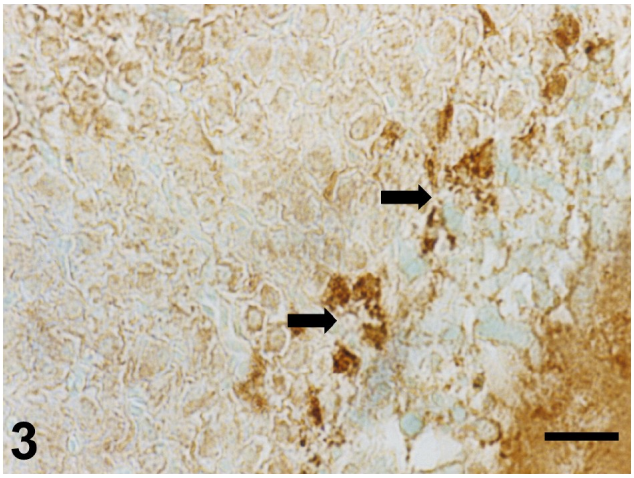

Figure 3 : TUNEL assay of junvile common carp (*Cyprinus carpio*) at 24 hr after intraperitoneal injection of 40 mg/L gallium. A small number of TUNEL-positive cells are scattered in the liver (black arrows). (bar = 0.15 mm)

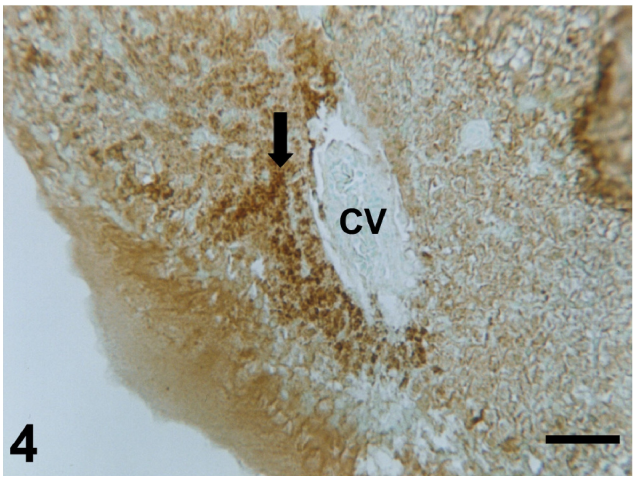

Figure 4 : TUNEL assay of junvile common carp (*Cyprinus carpio*) at 36 hr after intraperitoneal injection of 40 mg/L gallium. Strong TUNEL-positive cell staining around the central vein (black arrow) (CV) in the liver. (bar = 0.15 mm)

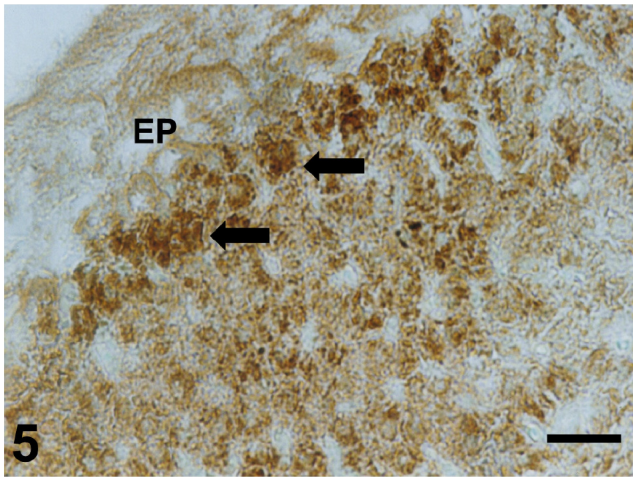

Figure 5 : TUNEL assay of junvile common carp (*Cyprinus carpio*) at 36 hr after intraperitoneal injection of 40 mg/L gallium. A large amount of TUNEL-positive cell staining is located on the margin of the neighboring liver epithelium (EP) (black arrows). (bar = 0.15 mm)

hr-group (Figure 2), but a few positive hepatocytes were scattered in the liver of fish after 24 hr antimony administration (Figure 3). Cellular changes increased in severity with time. On 36 hr-group, positive cell staining, nuclei, and smaller cellular fragments were diffusely scattered around the central vein in the liver of fish (Figure 4). Further, strong positive cells staining were found on the margin of the epithelium near the liver of fish.

DISCUSSION

The determination of the critical oxygen concentrations for regulation of oxygen consumption provides important information on the physiological condition of the aquatic organism[9]. After 30 mins, increased oxygen consumption in exposed fish was observed in all exposed groups. Murty[2] indicated that exposure to sublethal toxicant concentrations increases respiratory activity, resulting in increased ventilation, and increased uptake of the toxicant. Later, the decrease in oxygen consumption by common carp in the presence of toxicant can possibly be attributed to injury to the gills. Karan et al.[15] indicated that cytological damage occurs in common carp exposed to heavy metal, and it may manifest as a thickening of the branchial epithelium and increase the pollutant-blood diffusion distance, causing

impaired gaseous exchange. This kind of change might have occurred in common carp upon exposure to gallium. A slight decrease in oxygen consumption was seen in common carp from days 0 (30 min) to 14 day of the exposure period for higher exposure level groups (4.0 and 8.0 mg/L). Common carp showed oxygen consumption recovery after 1-week period in antimony-free water. Some studies reported that during exposure to sublethal concentrations of heavy metal, the above condition was reversed after 14 days in common carp[15] and 30 days in *Cancer pagurus*[7] even in the continued presence of the toxicant. A comparison study of the responses of fish under minimal dissolved oxygen and gill morphological adaption[16], mentioned that aquatic life possess an excellent regulatory ability in their oxygen consumption patterns. Our results suggest that measurements of oxygen consumption can be used to assess the effects of gallium on sublethal exposure levels of common carp.

A histochemical location assay was carried out on a liver of gallium-treated common carp. The result revealed a large number of darkly stained cell nuclei in the 36hr-exposure group under light microscopy. Pathologically, a TUNEL positive reaction can appear in both necrosis and apoptosis[11]. This led us to further investigate ultrastructural change in positive cells on the basis of morphological appearances. Consequently, the adverse effect was proposed us that fish treated with the gallium further supporting the possibility of developing fish modeling for the study of toxicology.

All of our findings support gallium being a potential pollutant to common carp, producing alterations in respiratory effects at higher exposure concentrations, with more damage as the gallium concentration increased. Also can provide important information in terms of toxic effects of aquatic pollution and be used as an early warning system. This and further studies in these fields will be helpful to clarify how fish react to gallium stress, although no adverse effects following industrial exposure have been reported to date.

ACKNOWLEDGMENTS

We wish to thank Dr. CT Lee (Hematology Division, Chang Gung Memorial Hospital) for technical assistance with the blood smears; and Mr. Chamberlin for correction of the English text.

REFERENCES

[1] A.L.Menke, J.M.Spitsbergen, A.P.M.Wolterbeek, R.A.Woutersen; Toxicol. Pathol., **39**, 759-775 **(2011)**.
[2] A.S.Murty; Toxicity of Pesticides to Fish, CRC Press; New York, **2**, **(1986)**.
[3] D.Jacobson-Kram, K.A.Keller; Toxicology testing handbook', Marcel Dekker; New York, **(2001)**.
[4] D.R.Webb, S.E.Wilson, D.E.Carter; Am. Ind. Hyg. Assoc. J., **48**, 660-667 **(1987)**.
[5] J.A.Sturgill, J.T.Swartzbaugh, P.M.Randall; Clean Prod. Proc., **2**, 18-27 **(2000)**.
[6] J.Bustamante, D.Lennart, V.Marie, F.Bruce, O.Sten; Toxicology, **118**, 129-136 **(1997)**.
[7] J.I.Spicer, R.E.Weber; Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol., **100**, 339-342 **(1991)**.
[8] J.L.Yang, H.C.Chen; Bull. Environ. Contam. Toxicol., **71**, 240-247 **(2003)**.
[9] J.P.Wu, H.C.Chen; Chemosphere, **57**, 1591-1598 **(2004)**.
[10] K.P.Hoyes, R.C.Hider, J.B.Porter; Cancer Res., **52**, 4591-4599 **(1992)**.
[11] M.Leist, P.Nicotera; Biochem. Biophys. Res. Commun., **236**, 1-9 **(1997)**.
[12] M.Omura, A.Tanaka, M.Hirata, M.Zhao, Y.Makita, N.Inoue, K.Gotoh, N.Ishinishi; Fundamen. Appl. Toxicol., **2**, 13-26 **(1996)**.
[13] P.Cenini; J. Zool. Lond., **204**, 509-520 **(2000)**.
[14] U.H.Riaz, J.P.Wereley, C.R.Chitambar; Exp. Hematol., **23**, 428-432 **(1995)**.
[15] V.Karan, S.Vitorvic, V.Tutundzic, V.Poleksic; Ecotox. Environ. Safe., **40**, 49-55 **(1998)**.
[16] V.Matey, F.Iftikar, G.De Boeck, G.R.Scott, K.A.Sloman, V.M.F.Almeida-Val, A.L.Val, C.M.Wood; Can. J. Zool., **89**, 307-324 **(2011)**.
[17] W.Burggren, J.Roberts; Respiration and metabolism, in C.L.Prosser, 'Environmental and metabolic animal physiology', Wiley-Liss, New York, 353-435 **(1991)**.
[18] Y.Aoki, M.M.Lipsky, B.A.Fowler; Toxicol. Appl. Pharmacol., **106**, 462-468 **(1990)**.
[19] Y.Gavrieli, Y.Sherman, S.A.Ben-Sasson; J.Cell Biol., **119**, 493-501 **(1992)**.