Bull. Environ. Contam. Toxicol. (2002) 68:817–823

DOI: 10.1007/s00128-002-0028-3

Bulletin of Environmental Contamination and Toxicology

Acute Immunotoxicity of Gallium to Carp (*Cyprinus carpio* L.)

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Received: 10 August 2001/Accepted: 31 December 2001

In many years, gallium has seen increased used for integrated circuits in semiconductor industry. In this case, gallium dust emissions are relatively high (Fowler and Dobrota, 1989). According to Chepesiuk (1999), the manufacture of semiconductor technology using gallium can produce negative consequences for the environment. Actually, no levels of tolerance have been established for gallium and the extent of pollution caused by this industry is not yet evaluated (Hayes, 1988). It is then useful to assess and predict the potential risk of gallium for aquatic ecosystems. Fish are one of the most important indicators of environmental contamination of water and some of their physiological functions as immune system are effective bioindicators of water quality (Fournier et al., 2000). The integrity of the immune system is fundamental to good defense against a variety of pathogenic agents in the environment. Metal ions released into aquatic systems have been recognised as stressors causing immunosuppression in fish resulting in increased succeptibility to infectious diseases (Zelikoff, 1993). The present study reports the lethal toxicity of gallium for common carp and the acute and sublethal effects on some of their immune parameters in term of ecotoxicological risk assessment.

MATERIALS AND METHODS

Common carp weighing 25 ± 3 g were purchased from a local hatchery (Ets « Au Vairon » - Reims, France) and were placed in aerated spring water (Ca^{2+}, 98 $mg.l^{-1}$; Mg^{2+}, 4 $mg.l^{-1}$; Na^{+}, 4.1 $mg.l^{-1}$; K^{+}, 1.9 $mg.l^{-1}$; HCO_3^{-}, 269 $mg.l^{-1}$; SO_4^{2-}, 43 $mg.l^{-1}$; Cl^{-}, 3.6 $mg.l^{-1}$; NO_3^{-}, <2 $mg.l^{-1}$) (Aurele, Ardennes, France) with a photoperiod 12 h : 12 h. Fish were allowed to acclimatize for 3 weeks and were fed twice daily with commercial pellets (Tetramin™, Germany) before the start of the experiment. The condition of the fish were judged to be excellent, based on the absence of disease and mortality. The lethal toxicity experiments were conducted for 96 h in static conditions . Carp were maintained in gallium containing water (5, 10, 25, 50, 100, 250 or 500 $mg.l^{-1}$). Each tank (80 l) was stocked with 20 fish. The cumulative mortality was recorded every 12 hours and the median lethal concentrations at 96 h was determined using the probit method analysis (Litchfield and Wilcoxon, 1949) using StatPhar 2.4 Software for MS-DOS. The same experiment was realised in triplicate. A total of 60 fish was then

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exposed to each test concentration. In tanks, the water quality parameters (dissolved oxygen, temperature, pH,...) were determined daily until the end of the experiment according to Standard Methods for the Examination of Water and Wastewater (APHA, 1989). The measured values for water quality parameters during the exposure period were similar to those measured during acclimation (temperature, 17 ± 2 °C ; dissolved oxygen, 8.45 ± 0.4 $mg.l^{-1}$; pH, 7.1 ± 0.07 ; ammonia, 0.003 ± 0.001 $mg.l^{-1}$). For experiments, gallium nitrate ($Ga(NO_3)_3$, Sigma, France) was dissolved in water to obtain a stock solution of Ga^{3+} (3 $g.l^{-1}$) which was added in tank to the dilution water to produce the test concentrations. The real Ga^{3+} concentrations in water were monitored using atomic absorption spectrophotometry (SpectrAA-20, Varian, Australia). For determination of gallium effects on immune related parameters, we chose a lethal (50 $mg.l^{-1}$) and a sublethal (5 $mg.l^{-1}$) exposure levels of gallium. They were calculated as 5 and 50 % of estimated 96 h LC_{50} value of gallium to carp. For each concentration, twenty fish were stocked in 80 l tank. The acute intoxication experiment was run for 96 h to examine the mechanisms underlying the acclimatation to the toxic effects of the metal. At different times (0, 24, 48 or 96 h), fish were sacrified and bled from the caudal vein using an heparinized syringe to isolate blood leucocytes and plasma (Rowley, 1990). At the same time, phagocytes were isolated from head kidneys (Secombes, 1990). The Lowry et al. (1951) method was followed to measure the total plasma protein. The immunoglobulin levels and total plasma lysozyme activity, a bacteriolytic enzyme, were evaluated with a procedure of Anderson and Siwicki (1993). The number of viable isolated leucocytes was counted by the Trypan blue exclusion test (Sigma, France) using the method described by Rowley (1990). The potential killing activity of head kidney phagocytes was estimated by the chemiluminescence assay (Secombes, 1990). For statistical analysis, differences in means were analyzed by one-way ANOVA in conjuntion with Dunnet's test using the software SigmaStat for MS Windows version 2.03.

RESULTS AND DISCUSSION

The mean LC_{50} of gallium for 96 h exposure were determined as 95.6 ± 14.3 $mg.l^{-1}$ (Table 1). Lin and Hwang (1998) estimated the 96 h LC_{50} of gallium for tilapia (*Oreochromis mossambicus*) larvae to be 36 $mg.l^{-1}$. For comparison 96 h LC_{50} for Zn^{2+} and for Cu^{2+} were 25 $mg.l^{-1}$ and 1 $mg.l^{-1}$ respectively in cyprinidae (Leland and Kuwabara, 1985). Gallium seems to be a low potential toxicant for carp in comparison with tilapia larvae or with other classical metal ions. Measured gallium concentrations in water were closely approximated nominal value (Table 2). They were relatively constant throughout the exposure time inspite of a low unsignificant decrease observed. Gallium seems to be relatively persistant in water. Actually, no data were available concerning effects of gallium on fish and especially on their immune system. Head kidney phagocytes have an important role in regulating nonspecific and specific immune responses in fish (Secombes and Fletcher, 1992). When carp were maintained in gallium containing water (50 $mg.l^{-1}$), the killing activity of head kidney phagocytes was decreased especially at 48 and 96 h exposure (Figure 1a). No modulation was observed for fish exposed to 5 $mg.l^{-1}$ gallium whatever the exposure duration might be (Figure 1a). Change

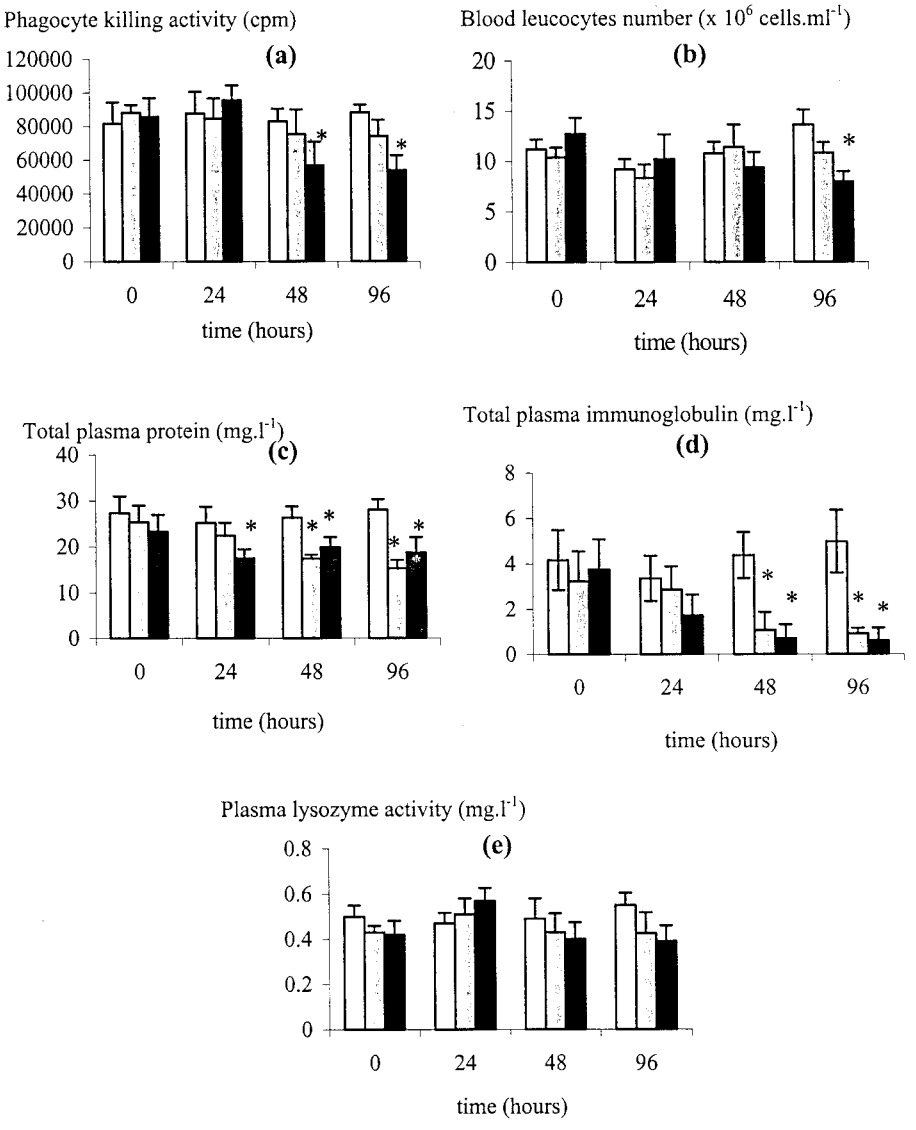

Figure 1. Immune related parameters measured in gallium-exposed carp. (□), 0 (control) ; (▩) 5 mg.l^{-1} (■) 50 mg.l^{-1}. Bars were means ± SE (n=10). For each exposure duration, asterisks denote significant differences from the respective control (0 mg.l^{-1}) at $p<0.05$ (*) and $p<0.01$ (**).

Table 1. Test concentrations and mortality of carp exposed to gallium in water for 96 h.

Average measured concentrations ($mg.l^{-1}$)	Mortality (numbers of dead fish)					
	n	24 h	48 h	72 h	96 h	Total (%)
0	60	0	0	0	0	0
4.7	60	0	0	0	0	0
9.6	60	0	0	0	0	0
23.4	60	0	0	0	0	0
49.3	60	6	3	1	0	17
95.2	60	13	9	4	2	47
240.5	60	23	25	5	5	97
489.0	60	60	0	0	0	100

in phagocyte respiratory burst activity was then observed only in carp exposed to a lethal gallium concentration (50 $mg.l^{-1}$). Moreover, only carp maintained in 50 $mg.l^{-1}$ gallium-containing water for 96 h had a significantly lower number of blood leucocytes than controls maintained in normal springwater (Figure 1b). Only at the highest concentration in water (50 $mg.l^{-1}$), gallium was cytotoxic for fish blood leucocytes. In mammals, there are many examples of the cytotoxic action of gallium particularly against tumorals cells (Bernstein, 1998). Our results suggest that the exposure of carp to the highest and lethal gallium concentration used (50 $mg.l^{-1}$) influences the potential killing activity of phagocytes and reduce the blood leucocyte number. The immune defense of carp against pathogens is then reduced. Moreover, we showed here that total plasma protein and immunoglobulin levels were significantly decreased in plasma of gallium-exposed carp (5 or 50 $mg.l^{-1}$) for 48 or 96 hours (Figure 1c and Figure 1d). Total plasma protein was already inhibited in gallium-treated carp for 24 h. However, no effect was observed on plasmatic lysozyme activity (Figure 1e). Thus, in case of an acute environmental contamination, high gallium levels in water are stable in time and gallium can be then implicated as an immunosuppressive metal ion in fish as already observed for other metal ions (Zelikoff, 1993). However, changes in immune parameters observed are probably due to decrease fish health and not to the direct action of gallium upon the immune system. Gallium-exposed carp may live under stressful conditions. According to Pickering (1993), the stress response can be regarded as a mechanism that enables fish to overcome potentially threatening or harmful situations. Even though the response may vary according to different circumstances, there appears to be a common element in response to all forms of environmental stress. This involves particularly the activation of the hypothalamic-pituitary-interrenal axis for secretion of corticosteroid hormones (cortisol...) into the circulatory system (Schreck, 1981). Moreover, as other metal ions, gallium can modulate the fish mineral balance. Metal ions affect gill functions, thus inducing the loss of critical ions (Reid and Mc Donald, 1991).

Table 2. Dissolved gallium concentrations in water.

Time (hours)	Nominal $[Ga^{3+}]$ in water ($mg.l^{-1}$)		
	0 (control)	5	50
	Gallium concentrations in water ($mg.l^{-1}$)		
0	not detectable	5.3 ± 1.3	50.7 ± 5.4
24	not detectable	4.8 ± 0.8	50.3 ± 3.6
48	not detectable	4.6 ± 1.1	48.5 ± 4.7
96	not detectable	4.5 ± 0.7	46.2 ± 3.4

Data were means ± SE (n=10). For ach exposure duration, asterisks denote significant differences from the respective control (0 $mg.l^{-1}$) at $p<0.05$ (*) or $p<0.01$ (**)

Cortisol, in addition to being a glucocorticoid, is a mineralocorticoid, threrefore cortisol levels rise to balance ion loss in the gills (Richman and Zaugg, 1987) This rising cortisol then affects immune functions (Maule and Schreck, 1990). Cortisol can stimulate or inhibit such activity, depending on the dose and the exposure duration. The immunotoxic effects observed in gallium-exposed carp were probably due to the indirect effect of the chemical according to this endocrine hypothesis. But these effects can be due to a direct action of gallium on immune response as observed in mammals where gallium directly reduces some immune functions too (Bernstein, 1998). For example, at high concentrations, it directly inhibits macrophage activation and suppresses cytokine secretion by these cells (Makkonen et al., 1995). The acute toxicity test showed here that gallium is a relatively low toxic chemical for carp in comparison with other metal ions. But as an immunotoxicological point of view, the acute sublethal effects of gallium on fish immune system were significant and were able to increase the sensibility of fish to pathogens (Anderson, 1990). Ensuing mortalities are then related to fish stress response associated with many physiological disturbances implicating nervous and endocrine systems but also the immune system., as observed here. This study conducted under short-term exposure conditions gives informations on the acute immunotoxic effects of gallium to carp. However, the effects of this metal ion on fish immunity might be important in case of chronic environmental contaminations and should be studied.

Acknowledgments We thank the Conseil Régional de Champagne-Ardenne (France) for its financial help in making this study possible.

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