Evaluation of acute toxicity of copper cyanide to freshwater fish, *Catla catla* (Hamilton)

Hosetti BASALING[1], Dube PRAVEEN

[1]Toxicology Division, PG Dept Zoology, Bioscience Complex, Jnana sahyadri, Kuvempu University, Shankaragatta- 57745, Shimoga, Karnataka State, India,e mail: basaling@yahoo.co.in, pndube_skyline@yahoo.co.in

ABSTRACT

The acute toxicity of copper cyanide to the juveniles of Indian major carp *Catla catla* (2±0.5 cm) was evaluated under static renewal conditions. The concentrations of copper cyanide that killed 50% of the carp fingerlings within 96-h (96-h LC_{50}) was found to be 0.76 ± 0.04 mg/L. Fishes exhibited abnormal swimming activity, followed by frequent gill flapping, loss of equilibrium, gulping of air, hyperactivity, convolutions. The respiratory rate of the fish got severely affected (-63.41%± 0.002) when exposed to copper cyanide..

Keywords: Behaviour, *Catla catla,* Copper cyanide, LC_{50}, Oxygen consumption

INTRODUCTION

Cyanide comprises a wide range of compounds, which are present in environmental matrices and waste streams as simple and complex cyanides, cyanates and nitriles [12], all of which contain CN moiety, a primary toxic agent. Despite its toxicity, many chemical forms of cyanides are used in industrial applications includes, metal-plating, mining, agricultural, and chemical industries and their effluents are the major sources of pollutants in the environment [17]. The release of cyanide from the industries worldwide has been estimated to be more than 14 million kg/yr [12]. Cyanide in receiving streams can result in fish kills either directly or indirectly by destroying the organisms upon which the fish feed. Fish and aquatic invertebrates are particularly sensitive to cyanide exposure [23].

Copper cyanide in an off-white to green colored inorganic compound with the formula CuCN and is ranked as one of the hazardous compounds (worst 10%) to ecosystems and human health [2]. It is used for electroplating of copper [25] and also used as a catalyst in polymerizations, and as insecticide, fungicide, and biocide in marine paints. The compound is useful reagent in organic synthesis, for example in the regioselective and stereoselective allylation and conjugate addition of N-Boc-2-lithiopyrrolidine and N-Boc-2-lithiopiperidine, [9] or the copper cyanide catalyzed palladium coupling of α-lithio amines and aryl iodides [10].

Copper cyanide is insoluble in water but rapidly dissolves in solutions containing CN^- to form $[Cu(CN)_3]^{2-}$ and $[Cu(CN)_4]^{3-}$, these ions are triagonal planar and tetrahedral respectively. This is in contrast to both silver and gold cyanides which

form $[M(CN)_2]^-$ ions in solution [26]. The coordination polymer $KCu(CN)_2$ contains $[Cu(CN)_2]^-$ units which link together forming helical anionic chains [16]. It is also soluble in 0.88 aqueous ammonia, pyridine and N-methylpyrrolidone and other alkalis. Although by themselves, it is much less toxic than free cyanide; its dissociation releases free cyanide as well as the metal cation which can also be toxic. Even in the neutral pH range of most surface water, copper cyanide complex can dissociate sufficiently to be environmentally harmful if in high enough concentrations [1]. Stability of copper cyanide depends upon the pH of water. and therefore, the potential environmental impacts and interactions (i.e. their acute or chronic effects, attenuation and re-release) can vary [4].

Behavioural changes in animals are indicative of internal disturbances of the body functions such as inhibition of enzyme activities [5], impairment in neural transmission, and disturbances in metabolic pathways [13]. The elimination of aquatic animals by small insidious physiological or behavioural changes has been reported to be more serious than a massive fish kill, since it is less likely to be observed and corrected [6]. Variation in the respiratory rate of animal is an indicator of stress, which is frequently used to evaluate the altered metabolism under environmental deterioration. Oxygen consumption is widely considered to be a critical factor for evaluating the physiological response and useful variable for an early warning for monitoring aquatic organisms [8]. Like most fish, Indian major carp (*C. catla*) are oxygen regulators, i.e., they maintain their oxygen consumption at a constant level along a gradient of environmental oxygen concentrations, until critical oxygen concentration is reached (< 60% water saturation), 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 contaminations.

Fish are one of the most cyanide sensitive organisms. Indian major carp *C. catla* is an important cultured fish species in fish ponds due to its fast growth and economic value. Nonetheless studies on the toxicity of copper cyanide on the fishes are scanty despite several decades of its introduction, wide use and magnitude. Therefore, the present study was designed to investigate the acute toxicity and effects of copper cyanide on the behaviour and respiratory activity in the carp (*C. catla*).

MATERIALS AND METHODS

Healthy fingerlings of *C. catla* (length 2±0.5 cm; weight 1.5±0.2 g) were obtained from State Fisheries Department, B.R. Project, Shimoga, Karnataka, India and reared in large cement tanks for a period of 15 days (2g/L). Prior to stocking, fishes were treated with 1% $KMnO_4$ to remove any dermal adherent. During acclimatization, the fish were fed with rice bran and oil cake in the ratio of 3:1 on alternate days. Water of the tank was changed daily to avoid any fungal and bacterial contamination. In the laboratory the fishes were maintained in large aquaria of 100 L capacity (100 x 35 x 50 cm). Dechlorinated tap water was used during the entire period of acclimatization and test periods, whose physico-chemical characteristics were analyzed following the

methods mentioned in APHA [1] and were found as follows, temperature 27±1 ºC, pH 7.4±0.2 at 27ºC, dissolved oxygen 6.8±0.5 mg/L, carbon dioxide 6.3±0.4 mg/L, total hardness 23.4±3.4 mg as $CaCO_3$/L, phosphate 0.39±0.002 µg/L, salinity 0.01ppm, specific gravity 1.001 and conductivity less than 10 µS/cm. Water was renewed every day and a 12-12 h photoperiod was maintained during acclimatization and test periods. In the laboratory, fish were fed regularly with commercial fish food pellets, (Nova, Aquatic P. Feed, not less than 3% of their wet body weight) and feeding was stopped two days prior to exposure to the test medium for acute toxicity test.

Acute copper cyanide (97% purity, Loba chemicals, Mumbai, Maharashtra) toxicity tests were conducted with C. catla according to APHA guidelines [1]. Total of 100 fishes were selected and were divided in to batches of ten each, and were exposed to different nominal copper cyanide test concentrations (0.58, 0.62, 0.70, 0.76, 0.86 mg/L), and three replicates were maintained for each concentration, which resulted in the mortality of the fish within the range of 0 to 100% in the range finding test, obtained on trail and error basis. Simultaneous control group was also maintained together with the actual experiments. The toxicant solution in the test and control chamber (20L) was replaced with the fresh solution of the same concentration every 24 hours. Observations were made at every 24 h, and immobilized organisms for 15 s after gentle shaking and a change to milky coloration were considered dead and the number of which was recorded. Dead animals were removed at each observation time. Acute toxicity tests were carried out for a period of 96 h [1]. The mortality rate was determined at the end of 24, 48, 72 and 96 h and dead fish were removed as and when observed. The concentrations in which 50% mortality (LC_{50}) occurred were obtained graphically by probit analysis, plotting concentration against fish mortality [14]. During the experimental period the control and copper cyanide exposed fish were kept under constant observation to study behavioral abnormalities. The responses of the fish to the toxic medium was assessed in the form of change in the swimming pattern, group dynamics, avoidance to the test medium, altered opercular movements as mentioned by Kumari et al [19].

To estimate the oxygen consumption the respirometers were filled up with solutions of copper cyanide in the same concentrations of those used in the experiments. Before the beginning of the experiments, the animals were maintained in the respirometers with continuous water circulation for at least 90 minutes to attenuate the stress caused by handling. Then, the water supply was suspended and the respirometer was closed, so that the fish could consume the oxygen present in a known volume of water for a period of an hour. Only one fish was used in each respirometer. The respirometers were protected by a shield to isolate the animals from possible external disturbances in the laboratory. The difference between the concentrations of oxygen determined at the beginning and at the end of the confinement was used to calculate the oxygen consumption per animal. To minimize the effect of low oxygen concentration and the metabolites accumulation in the organism, the duration of the experiments was regulated so that the oxygen concentration at the end of experiments was larger than the 70% of its initial concentration. To obtain the desired concentration of copper cyanide, the required volume of the stock solution was calculated for the volume in each respirometer and

set with a micropipette at the end of the acclimation. As soon as the toxicant was added, the entry orifice of the respirometer was sealed. Oxygen consumption was determined by using Winkler's iodometric method as mentioned by Chebbi and David [7] and the values are expressed as ml of oxygen consumed/g wet wt. of fish/h.

RESULTS

The fish of the control groups were observed to be healthy, normal and active with well coordinated body movements and alert to slightest disturbance. No mortality was recorded in the control group. Concentrations of the test compound used in short term definitive tests were between the highest non-lethal concentration at which there was 0% mortality and the lowest lethal concentration at which there was 100% mortality (Fig. 1). The results obtained from 96 h toxicity experiments for copper cyanide for the carp and the estimated 96 h LC_{50} value and confidence limits are listed in Table 1. The mean LC_{50} value of copper cyanide to the juveniles of *C. catla* was found to be 0.76 ± 0.04 mg/L Thus, copper cyanide can be rated as highly toxic to fish.

Table 1: Acute toxicity (96 h LC50), regression equation, and 95% confidence limits of copper cyanide to the fingerlings of C. catla.

Toxicant	Regression equation $y = mx + c$	96 h LC50 (mg/L)	95% Confidence limits	
			Upper limit	Lower limit
Copper cyanide	y = 533.25x + 126.21	0.76 ± 0.04	0. 812	0.735

The carp fingerlings exhibited a number of abnormalities in their behavior when exposed to copper cyanide. Within a two hours of exposure up to 96 h LC_{50} (0.76 mg/L), the fishes appeared excited, the swimming became erratic and the schooling was disrupted followed by hyperactivity, loss of balance, increased surfacing, enhanced rate of opercular activity and convolutions. The mucous secretion increased considerably in exposed fishes followed by increase in the defecation at the bottom of the test chamber when compared to control fishes. The exposed fishes exhibited tremors and gradual weakening of reflexes leading to imbalance in posture and loss of balance. Fishes started drowning but by sudden somersaulting, regain normal posture and balance temporarily. Body color changed from silvery white to pale white, with copious secretions of mucous all over the body (after 72 h of exposure). At the time of death they exhibited transient hyperactivity before collapsing (Table 2).

Table2: Effect of Copper cyanide (0.76 mg/L) on the behavioral patterns of C. catla at different time intervals

Parameters	Control	Copper cyanide (0.76 mg/L)			
		24	48	72	96
Erratic swimming	-	++	+	++	+
hyperactivity	-	+	+	+++	++
Surfacing activity	-	++	+	+	+++
Convolutions	-	++	-	++	++++
Upside down swimming	-	-	-	+	++
Loss of balance	-	+	+	++	++++

The increase or decrease in the level of behavioral parameters is shown by numbers of (+) sign. The (-) sign indicate normal behavioral conditions

The results of the oxygen consumption rates for the control and exposed carps were presented in Table 3. The percent oxygen consumption rate decreased significantly at all exposure periods over the control fish; a maximum decrease of 63.41% ($p<0.01$) was observed on the 96 h of exposure to LC_{50} (0.76 mg/L) to copper cyanide.

Table 3. Oxygen consumption of the fish, C. catla following exposure to 96 h LC50 (0.76 mg/l) of Copper cyanide (ml of oxygen consumed/g wet wt. of fish/h).

Estimations	Control	Exposure periods in hours			
		24	48	72	96
Oxygen consumption	0.5519	0.4479*	0.3563**	0.2949**	0.2019**
±SD	0.003	0.0029	0.0031	0.005	0.002
% Change	------	-18.84	-35.44	-46.56	-63.41

Values are means ± SD (n = 5), significantly different from the control (*$p<0.05$, **$p<0.01$).

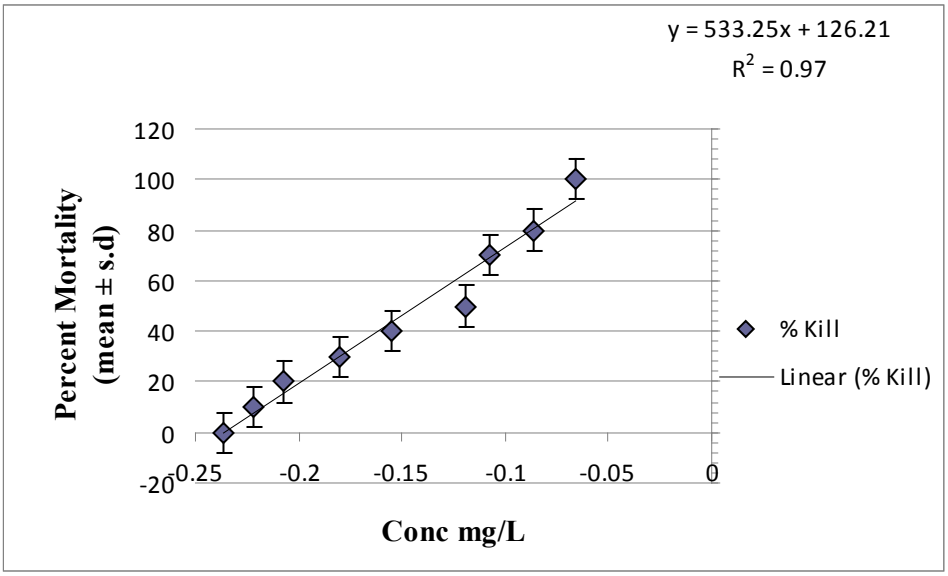

Fig. 1. Mortalities of Catla catla exposed to different concentrations of Copper cyanide at 96 h exposure period

DISCUSSION

The assessment of toxicity of copper cyanide with reference to aquatic biota, especially fish is crucial in establishing the toxicity evaluation. Mortality of fish due to copper cyanide exposure mainly depends upon its sensitivity to the toxicant, its concentration and duration of exposure. Reports on the acute toxicity tests by earlier workers revealed a clear idea of comparative lethal values of metal cyanides for different fish species. For 96 h treatment period, LC_{50} value of copper cyanide to the fish *Labeo rohita* was found to be 1.10 mg/L, zinc cyanide to *Labeo rohita* 0.35 mg/l [15] and *Cirrhinus mrigala* was found to be 0.343 mg/ l [28], sodium cyanide to *L. rohita* 0.32 mg/L respectively, where as in the present study the acute toxicity of copper cyanide to *C. catla* was found to be 0.76 mg/ L. On the basis of the observed 96h LC_{50} value, copper cyanide can be included in a group of substances that are highly toxic for fish: the risk sentence R50 states the values of 96h LC_{50} less than 1 mg/l [29].

Changes in fish behavior appear to be among the most sensitive and earliest indicators of environmental stress condition [28]. Behavioral data are useful as predictive indices of population and community-level effects because disruption of essential functions such as predator–prey relationships can become ecologically apparent through population changes when enough individuals are affected [21].

Behavioral changes observed in the exposed carp fingerlings, appear to be the manifestation of copper cyanide toxicity. Upon exposure to the toxicant, the immediate response of the fish is to drive to the bottom of the test chamber and stay there for a period of time. Diving to the bottom resembles the approach to nets and walls of tanks [22], which can also be interpreted as avoidance behaviour. Increase in

surfacing and gulping of surface waters appears to be an attempt by the fish to avoid breathing in the poisoned water. Moreover, hypoxic condition also contributes to increase surfacing as reported by Shwetha and Hosetti [28]. Approaching water surface was found in hypoxia-stressed fish [3, 18], as cyanide induces hypoxic condition. The increase in opercular movement and corresponding increase in rate of surfacing of fish clearly indicates that fish adaptively shifts towards aerial respiration (by obtaining atmospheric oxygen surfacing) and the fish tries to avoid contact with the cyanide through gill chamber. In all experiments, the fish reduced their swimming activity within the school. Reduced swimming speed due to hypoxia stress was found by Israeli and Kimmel [18], while Newton [24] reported reduced aggression in stressed fish. Fish exposed to copper cyanide slowly reached the water surface, probably due to gill damage that caused respiration malfunctions, or as a result of difficulties in gas exchange due to mucous accumulation. The same response was observed in hypoxia stress in carps [18]. Further, gill ventilation frequency is intimately associated with the respiratory demand and gill irritation or blockage, and locomotor activity may reflect a more specific stress response resulting, changes in blood cortisol and glucose levels [30, 27]. The examination of the gill of dead fish revealed that the gill lamellae colour was changed from red to brown. The unusual behaviour of the fish *C. catla* in stress condition may be due to obstructed functions of neurotransmitters. The increased opercular movements in the initial period of exposure might be to support enhanced physiological activities in stressful habitat and later decreased, may be due to accumulation of mucus over the gill filaments. The excessive secretion of mucus over the gills may inhibit the diffusion of oxygen during the process of gaseous exchange. These behavioural patterns are the indicative of respiratory impairment due to the effect of toxicant on the gills and general metabolism [28]. The reduction of respiratory rate implies that the fish had become fatigued due to several attempts to escape from the toxic medium to facilitate more oxygen intake.

One of the first symptoms of cyanide exposure is rapid gill flapping. Cyanide binds preferentially to the iron porphyrins present in cytochrome oxidase and prevent the normal utilization of oxygen by the tissues [20] thereby inhibiting the cellular respiration. Therefore oxygen, cannot react with hydrogen to form water in the cells, thus the concentration of hydrogen emerging from the metabolic pathways causes an acidosis in the cells [31]. Despite the regulatory capability of the fish exposed to the toxicant, the oxygen consumption rate was indeed decreased in copper cyanide exposed fish (Table 3). Downing [11] studied the influence of oxygen on the toxicity of KCN to rainbow trout and reported inverse relationship of cyanide to the oxygen consumption. Present study reveals the onset of acute hypoxia under stress. Reduced oxygen consumption at higher concentrations of cyanide could also arise as a result of respiratory inhibiting factors that come into play. The decrement in respiratory rate can also be attributed to the induction of hypoxic conditions in the animal due to the contact of the respiratory surface with the toxicant resulting in the alteration of normal respiratory area. Apart from behavioral changes, Respiratory distress and mortalities of fish reported in the present study, there have been no reports on the effects of copper cyanide on the nutritional value. Further studies are recommended on the nutritional status of fish to guard against associated health risks

from consumption of the contaminated animals. The endpoints in the present study are useful measures to assess copper cyanide toxicity in wild populations fish.

ACKNOWLEDGEMENTS

We wish to express our gratitude to University Grant Commission New Delhi, for the financial support in the form of Major Research Project (F.No.34-459/2008 SR) and to Kuvempu University for providing necessary infrastructural facilities to carry out the experiments. We also thank State Fishery Dept, BR Project, Shimoga, Karnataka, India for timely supplying of the fish fingerlings

REFERENCES

[1] APHA., AWWA., WPCP., Standard methods for the examination of water and wastewater. 21st ed. American Public Health Association, Washington, DC (2005).

[2] ATSDR (Agency for Toxic Substances and Disease Registry)., Toxicological profile for cyanide. Draft for public comment. Prepared for the U.S. Public Health Service by Technical Resources, Inc., under Contract No. 68-03-3268. Revised by Syracuse Research Corporation under Contract No. 68-03-3521. Oak Ridge National Laboratory (1998).

[3] Birtwell I.K., Kruzynski G.M., In situ and laboratory studies on the behavior and survival of Pacific salmon (genus *Oncorhynhus*). Hydrobiologia (1989) 188–189: 543–560.

[4] Caruso S.C., The Chemistry of Cyanide Compounds and Their Behavior in the Aquatic Environment, Carnegie Mellon Institute of Research (1975).

[5] Cearley J.E., Toxicity and bioconcentration of cadmium, chromium and silver in *Micropterus salmoides* and *Lepomis macrochirus*. Ph.D. Thesis. University of Oklahoma, Oklahoma City, OK. (1971) pp. 76.

[6] Chebbi S.G., David M., Behavioural and respiratory responses of the freshwater fish, *Cyprinus carpio* (Linnaeus) under quinalphos intoxication in sublethal tenures. Online. J. Vet. Res. (2009) 13 (1): 15-25.

[7] Chebbi S.G., David M., Respiratory responses and behavioural anomalies of the carp Cyprinus carpio under quinalphos intoxication in sublethal doses. Science Asia (2010) 36: 12–17

[8] Chinni S., Khan R.N., Yallapragada P.R., Oxygen consumption, ammonia-N excretion, and metal accumulation in *Penaeus indicus* postlarvae exposed to lead. Bull. Environ. Contam. Toxicol. (2000) 64:144–151

[9] Coldham I., Daniele L., “Regioselective and Stereoselective Copper(I)-Promoted Allylation and Conjugate Addition of N-Boc-2-lithiopyrrolidine and N-Boc-2-lithiopiperidine”. The Journal of Organic Chemistry (2010) 75 (12): 4069–4077.

[10] Dieter R.K., Sheng Jian Li., “Copper Cyanide Catalyzed Palladium Coupling of α-Lithio Amines and Aryl Iodides”. Tetrahedron Letters (1995) 36 (21): 3613–3616.

[11] Downing K.M., The influence of dissolved oxygen concentration on the toxicity of potassium cyanide to rainbow trout. Biochem F. (1953) 61-64.

[12] Ebbs S., Biological degradation of cyanide compounds. Biotechnol. (2004) 15: 1-16.

[13] Ellgaard E.G., Guillot J.L., Kinetic analysis of the swimming behaviour of bluegill sunfish, *Lepomis macrochirus* Rafinesque, exposed to copper: hypoactivity induced by sublethal concentrations. J. Fish Biol. (1988) 33: 601-608.

[14] Finney D.J., Probit Analysis, 3rd Edition, Cambridge University, Press, London. (1971) pp. 333.

[15] Hosetti B.B., Dube P.N., Prashanth M.S., Shwetha A., Acute toxicity of metal cyanides to Indian major carp *Labeo rohita* (Hamilton). Biotech in Animal Husbandry (2010) 26 (3-4): 267-277.

[16] Housecroft C.E., Sharpe A.G., Inorganic Chemistry (3rd ed.), Pearson: Prentice Hall (2008).

[17] IPCS., Hydrogen cyanide and cyanides: human health aspects. Geneva, World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment Document No. 61) (2004).

[18] Israeli D., Kimmel E., Monitoring the behavior of hypoxia-stressed *Carassius auratus* using computer vision. Aquacult. Eng. (1996) 15 (6): 423–440.

[19] Kumari R., Singh R.K., Khanna Y.P., Sharma B., Carbofuran induced stress mediated syndromes in *Clarias batrachus*. In: Proceedings of international conference on industrial pollution control technology (1997) 113–119.

[20] Leduc G., Cyanide in water: toxicological significance. In: Weber, L.J. (Ed.), Aquatic Toxicology, vol. 2. Raven Press, New York (1984) pp. 153–224.

[21] Little E.E., Behavioral measures of environmental stressors in fish. In: Adams, S.M. (Ed.), Biological Indicators of Aquatic Ecosystem Stress. American Fisheries Society, Bethesda, MD, (2002) pp. 431–472.

[22] Martinez Cordero F.J., Beveridge M.C.M., MUIR J.F., A note on the adult Atlantic halibut *Hippoglossus hippoglossus* L. in cages. Aquacult. Fish. Manage. (1994) 5: 475–481.

[23] McKee J.E., Wolf H.W., Water Quality Criteria, 2nd Ed. Sacramento, California State Water Quality Control Board (1963).

[24] Newton B.J., Early stress effects on growth and adult behavior in *Poecilia reticulata*. Dev. Psychobiol. (1982) 15(3): 211–220.

[25] Richardson W.H., "Copper Compounds" in Ullmann's. Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim (2005).

[26] Sharpe A.G., The Chemistry of Cyano Complexes of the Transition Metals. Academic Press. pp. 265 (1976).

[27] Scherer E., Harrison S.E., Brown S.B., Locomotor activity and blood plasma parameters of acid exposed lake whitefish (*Coregonus clupeaformis*). Canadian J Fish. Aqua. Sci. (1986) 43: 1556-1561.

[28] Shwetha A., Hosetti B.B., Acute effects of zinc cyanide on the behaviour and oxygen consumption of the Indian major carp *Cirrhinus mrigala*. World Journal of Zoology (2009) 4(3): 238-246.

[29] Velisek J., Wlasow T., Gomulka P., Svobodova Z., Dobsikova R., Novotny L., Dudzik M., Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). Veterinarni Medicina (2006) 51(10): 469–476.

[30] Wedemeyer G.A., McLey D.J., Methods for determining the tolerance of fishes to environmental stressors. In: Pickering AD(ed) Stress and Fish. Academic Press, London (1981) pp 247.

[31] Weger N.P., Treatment of cyanide poisoning with 4-dimethylaminophenol (DMAP): Experimental and clinical overview. Fund. Appl. Toxicol. (1983) 3: 387-96.