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Effects of sub lethal concentration of Chloramin T on growth, survival, haematocrit and some blood biochemical parameters in common carp fry (*Cyprinus carpio*)

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Abstract. This study was done in Gorgan University of Agricultural Sciences and Natural Resources, in 2009, during 8 weeks to survey effects of different concentrations of Chloramin T on fry common carp (*Cyprinus carpio* (Linnaeus, 1758)). According to the pre experiment data, lethal concentration, the lowest observed effect concentration, maximum allowable toxicant concentration and No Observed Effect Concentration of Chloramin T (Halamid) in common carp (*C. carpio*) fry were respectively 40.9, 27.1, 4.90 and 11.28 mg/L^{-1}. Hence, the range of our experiment was between 0 to 25 mg L^{-1} which was divided to five treatments (0, 5, 10, 15 and 25 mg/L^{-1}). At the end of experiment we calculated growth factor (special growth rate and food conversion ratio) and stress indices (glucose total protein and percent of haematocrit) and then they were compared with the controlled group. Our study results showed no significant difference between percentage of increase in body weight (280.4±25.79 - 200.4±10.16), special growth rate (2.34±0.25 - 1.96±0.06) and food conversion ratio (0.84±0.05 - 0.60±0.12) in experimental lots of fishes. There is also no significant difference between glucose (146.82±0.79 - 99.54±1.89) and total protein (3.61±0.45 - 2.82±0.06) in experimental groups (P>0.05). However there is a significant difference between the percentages of haematocrit (57.34±4.99 - 40.74±2.17) and cholesterol (362.05±24.38 - 134.92±17.59) in these groups (P<0.05).

Key Words: growth, survival, stress indices, Chloramin T, common carp.

خلاصه. این تحقیق به منظور بررسی اثرات زیر حد مرگ کلرامین **T** (هالامید) روی رشد، بقاء، هماتوکریت و برخی شاخص های بیوشیمیایی خون بچه ماهی کپور معمولی ((*Cyprinus carpio* (Linnaeus, 1758) در مرکز تحقیقات آبزی پروری دانشگاه علوم کشاورزی و منابع طبیعی گرگان در سال 1388 به مدت 8 هفته صورت گرفت **.** بر اساس نتایج پیش آزمایش غلظت کشنده، حداقل غلظت موثره، حداکثر غلظت مجاز و غلظت غیر موثر کلرامین **T** در بچه ماهی کپور معمولی به ترتیب برابر 40/9، 27/1، 4/90 و 11/28 میلی گرم در لیتر بود. در نتیجه دامنه آزمایشی ما بین 25-0 میلی گرم در لیتر قرار گرفت که به 5 تیمار (0، 5، 10، 15، 25 میلی گرم در لیتر) تقسیم گردید. در انتهای آزمایش میزان شاخص های رشد و استرس محاسبه و با گروه شاهد مقایسه گردید. نتایج به دست آمده نشان داد که مابین درصد افزایش وزن بدن (280/4±25/79 – 200/4±10/16) ، ضریب رشد ویژه (2/34±0/25 – 1/96±0/06) و ضریب تبدیل غذایی (0/84±0/05 – 0/60±0/12) در بین گروه های آزمایشی تفاوت معنی داری وجود ندارد. همچنین بین میزان گلوکز (146/82±0/79 – 99/54±1/89) و پروتئین کل (3/61±0/45 – 2/82±0/06) اختلاف معنی داری مشاهده نشد (**P>0/05**). اما بین درصد هماتوکریت (57/34±4/99 – 40/47±2/17) و کلسترول (362/05±24/38 – 134/92±17/59) در بین گروه های آزمایشی اختلاف معنی داری وجود داشت (**P<0/05**).

کلمات کلیدی. رشد، بقاء، شاخص های استرس، کلرامین **T** ، کپور معمولی .

Introduction. Culture of fish in dense farms causes stress on them and accordingly will have adverse effects on the growth process and physiological status. Furthermore, stress can reduce the resistance of fish to unfavorable environmental conditions and diseases (Asgharzade 2008).

One of the strategies to increase the animal resistance to stressful conditions and to improve their growth is improving conditions of captured fishes by changing some living and non-living components of their growing environment which can be called environmental disinfectants. A lot of researches have been done so far to minimize stress and its effects on fishes by using some compounds such as acetyl salicylic, biocides, or veterinary medicinal products. However, such materials are relatively expensive and their uses also show side effects. Therefore it is necessary to replace these compounds with a compound which is cheaper, easy to use and has fewer side effects.

Common carp (*Cyprinus carpio* (Linnaeus, 1758)) is one of the most important fishes in farm culture and due to being economical and because of its delicious meat, this fish has a special importance in many countries (Vosoghi & Mostajir 1986). The common temperature, PH, hardness and oxygen of water ponds for carps are respectively 15-60 ^{O}C, 8-6.5, 20 mg L^{-1} Ca_2Co_3 and more than 5 mg L^{-1} (Peighan & Mashaea 2005; Boyd 1998; Zweig et al 1999).

Chloramin T, with the chemical name of Sodium-Chloro-para-Toluenesulfonamide which is known on the market as Halamid, has been recently presented against the pathogenic organisms in fishes and shrimps. This substance dissolves easily in water. Both general and specific disinfections by Halamid in aquaculture industry assure that pathological microorganisms (bacteria, viruses, fungus, and parasites) disappear effectively and quickly. Remedial application of Halamid contains using it against pathogenic bacteria such as Bacterial Gilles Disease (BGD) and also against many of the parasites associated with the aquaculture industry such as *Gyroductilus* and amebic disease (Asgharzade 2008).

Fish production capacity can increase by identifying environmental factors and providing an appropriate environment for the fish. In addition, common carp is one of the important breeder species and if the optimum conditions required for the breeding of this fish is considered, this worthwhile fishery product can effectively improve and develop. Therefore this study was assigned to determine the lethal concentration of Halamid and to investigate the effects of sub lethal concentration of this substance on survival and growth factors, and biochemical and hematology parameters in the carp fry.

Material and Method. This study took place in the summer of 2009 in Central Laboratory of Agricultural Sciences and Natural Resources of Gorgan University. Tests were done according to standards of O.E.C.D (Organization for Economic Cooperation and Development Europe).

Based on pre-test results, desired concentration ranges of Lc_{50} were determined between 1 to 60 mg L^{-1}. This means that in concentrations more than 60 mg L^{-1} all fish had died after 24 hours. Hence, ranges were classified logarithmically to 10 treatments (1, 1.15, 2.28, 3.45, 5.21, 7.87, 11.28, 27.1, 40.9 and 61.8). Due to short time of the experiment and in order to prevent the environmental pollution, 24 hours before experiment fry were not fed and the environment (water) of experiment was not changed during this period (Javadi 1999).

The mean of that concentration of Halamid which killed 50% of fish population during 24 h of experiment was calculated as Lc_{50} 96h. Maximum Allowable Toxicant Concentration (MATC), is the concentration which does not have any deleterious effect on carp fry during the experiment, and which does not endanger their health. This number is calculated based on the proposed formula T.R.C. (1984) (Lc_{50} 96h divided by 10). In other words MATC is more than the concentration which has no effect on the experimental animals and is less than the concentration which has the least effect (Javadi 1999). No Observed Effect Concentration (NOEC) is the concentration which statistically does not show any significant effect on the experimental animals, compared with controlled group, which were exposed to this substance. Lowest Observed Effect Concentration (LOEC) is the concentration which statistically shows the least significant effect on the experimental animals exposed to the substance compared with the controlled group and according to some sources it equals to Lc_{10} 96h (NOEC <MATC <LOEC) (Rand 1995).

At the end of experiments fish were anesthetized by clove powdered and immolate. To measure percentage of haematocrit, the tubes containing blood were centrifuged at 3000 rpm for 8 minutes. Haematocrit reader (WPA Light Wave- Diode-array S2000 UV/Vis) was used to determine the percentage of haematocrit (Fitzpatrick et al 2005).

In order to study biochemical indexes of carp and to find regression relationship between biochemical indicators of serum, in different experimental groups, samples were put into the 1.5 mL micro tubes and these tubes were centrifuged in the 13,000 rpm for 5 minutes (Eppendorf AG 22311 Hamburg, centrifuge 5415D) and then they were transferred to new vials. The samples were stored in -20°C for future analysis. Concentrations of glucose, cholesterol and total protein were measured by the spectrophotometer (S200-UV.VIS England), using quantitative biochemical kits of serum or plasma parameters (Turker et al 2004).

Every 24 hours, the aquariums were checked and the amount of mortality was recorded (T.R.C. 1984); these records were done for 96 h, every day at the same time. Physical and chemical factors of the environment (water) were also recorded separately every 24 hours, for all treatments. During experiments, dead fishes were quickly picked up from the water and the condition and behavior of fish were recorded.

The temperature, pH, dissolved oxygen and hardness of water were mearsured by mercury thermometer, electronic pH meter, oxygen meter (oxi 330i wtw) and titration with EDTA respectively. Physiochemical factors of the aquarium water were measured and recorded daily.

After calculating Lc_{50}, specified concentrations for the main experiment were determined between 0 to 25 mg L^{-1} and then this range was divided into five treatments. For the main experiments 0, 5, 10, 15 and 25 mg L^{-1} of Halamid were used three times.

The carp fry, after weighing, were transferred to 12 aquariums, 20 fishes in each aquarium. Trials lasted for eight weeks. Considering the amount of food left on the bottom of aquarium, the amount of needed food was determined 3% of the fish body weight (level of fullness) and this proportion of food was given to fishes in three meals (8 am, 3 pm and 8 pm). During the experiments, by the growth of fish size, the amount of food (BioMar Ltd. France.) increased from 0.5 and 0.8 to 1.1 mm.

At the end of experiments, weight gain percentage (WGP), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated through the following formulas (Tacon 1990):

1. WGP: 100*((final weight- initial weight) / initial weight) [%]
2. SGR: 100*(ln (final weight) – ln (initial weight)) / no. of day [g%/day]
3. FCR: feed fed (g) / final weight (g) - initial weight (g) [g/g]

Percentage of haematocrit and blood stress indicators of carp fry was performed in accordance with methods mentioned in Lc_{50}.

Content of Lc_{10}, Lc_{50} and Lc_{90} was obtained by the Probit Analysis Statistical Method (Flik 1995).

Fish were distributed as a completely randomized design. Results of hematology and biochemical were analyzed by SPSS version 16. Comparing means were performed by analysis of variance ANOVA and Duncan multiple range tests and the presence or absence of statistically significant difference were determined at 5 % ($P<0.05$).

Percentages of hematocrit and blood stress indicators in carp were calculated according to methods mentioned in Lc_{50}.

Contents of Lc_{10}, Lc_{50} and Lc_{90} were calculated using Probit Analysis Statistical Method (Flik 1995).

Fishes were distributed quite randomly. Hematological and biochemical results were analyzed using SPSS version 16. Comparison between means were performed by analyzing the ANOVA variance and Duncan multiple ranges test and the presence or absence of significant statistical difference were determined at 5 % ($P<0.05$).

Results and Discussion. During experiments of Lc_{50} some physiochemical factors of water of aquariums were recorded. These records have been presented in Table 1.

Table 1

Physiochemical parameters of the water of experimental aquariums (LC50) (means ± S.D)

Factors	*Amount in aquarium*
Temperature (°C)	19±0.2
Dissolved oxygen (mg L^{-1})	>6±0.7
pH	7.5±0.5
Hardness (mg L^{-1})	300 - 410±0.0

Light conditions in experimental environment were 15D: 9L (9 hours in light environment and 15 hours in dark environment). For these parameters there were no significant difference between treatments (P<0.05).

The pH of water was increased with the increasing amonts of Halamid in each treatment. So that in concentration of 0 mg $L^{-1,}$ pH of water was 7 and in the concentration of 61.8 mg $L^{-1,}$ pH of water was 8.

The value of LC50 were shown in Table 2.

In this section, results of our experiments showed that in those carp fry with 2.5±0.17g weight, poisonous range of Halimid was determined between 27.10 to 60 mg L^{-1}.

According to our research the amounts of Chloramin T in common carp fry in L_{C10}, $_{50}$, $_{90}$, during 96 h, were respectively 61.8, 40.90 and 27.10 mg L^{-1}. In aquatic ecosystems or in the farms LOEC, MATC and NOEC of this substance were respectively 27.10, 4.90 and 11.28 mg L^{-1}.

Table 2

Effects of Chloramin T on mortality of carps fry in 2.5±0.17 g (Lc50) (means)

Experimental groups	*Concentration of Chloramin T* (mgL^{-1})	*Number of casualties*				*Remained fishes*
		24 h	*48 h*	*72 h*	*96 h*	*96 h*
control	0	0	0	0	0	20
1	1	0	0	0	0	20
2	1.51	0	0	0	0	20
3	2.28	0	0	0	0	20
4	3.45	0	0	0	0	20
5	5.21	0	0	0	0	20
6	7.87	0	0	0	0	20
7	11.28	0	0	0	0	20
8	27.10	0	2	0	0	18
9	40.90	2	4	3	1	10
10	61.8	6	8	3	1	2
11	100	20	20	20	20	0

Percentages of haematocrit and biochemical analysis of serum in blood of carp fry 2.5±0.17 g (L_{c50}) are shown in Table 3.

Our results showed significant difference between percentages of hematocrit and cholesterol in serums of experimental fish groups (P<0.05). However there was no significant difference between Total protein and Glucose in serums of above groups (P>0.05).

Overall behavior of fishes during 96 hours of experiments was observed in positions including excited, unusual behavior, abnormal secretions in the skin and body (mucus), slow movements and relatively complete stop (if these process continues it will lead to fishes death). Clinically responses and behavior of fishes in different concentrations of this substance were different. Fish fry which were exposed to high concentrations of Halamid immediately showed some symptoms such as restlessness, abnormal swimming and after some time corrosion of tails and fins and skin injuries. Also, these fishes moved motionlessly and gathered in the corner of aquarium. After that, fishes skin color faded gradually and also mucus increased and finally the state of lethargy, disordered breathing, abdominal swelling and in some cases exophthalmia and scoliosis status were observed. After that dead fishes were observed on the surface of water with swollen abdomens and soft tissues and downy bodies. In low concentrations, fish fry did not react noticeably in first hours but gradually they showed weak motions; however they reacted rapidly to physical stresses.

During main experiments some physiochemical factors of water of aquariums were recorded which have been presented in Table 4.

Table 3

Comparison between means of the percentages of haematocrit and amounts of glucose, cholesterol and total protein in serums of carp fry in different volumes of Halamid (L_{c50})

Experimental groups	*Concentration of Chloramin T (mgL^{-1})*	*The percentage of haematocrit (%)*	*Glucose ($mg\ dL^{-1}$)*	*Cholesterol ($mg\ dL^{-1}$)*	*Total protein ($g\ dL^{-1}$)*
control	0	40.58 ± 2.39[b]	32.42± 8.12	153.10± 20.30[cd]	3.49± 0.14
1	1	45.69 ± 0.22[ab]	36.55± 6.27	240.75± 27.69[a]	4.36± 1.52
2	1.51	51.59 ± 8.17[a]	36.97± 5.26	174.78± 9.93[bcd]	3.83± 0.33
3	2.28	41.75± 4.59[ab]	43.75± 7.09	194.09± 7.18[bc]	2.91± 0.46
4	3.45	45.37± 2.15[ab]	40.44± 4.52	168.17± 15.77[bcd]	3.21± 0.04
5	5.21	45.55± 5.72[ab]	56.71± 11.29	146.57± 0.26[d]	3.19± 0.49
6	7.87	45.00± 5.46[ab]	56.55± 6.77	170.05± 18.42[bcd]	3.52± 0.77
7	11.28	45.91± 1.06[ab]	44.44± 3.96	188.85± 38.45[bcd]	3.13± 0.38
8	27.10	49.69± 1.19[ab]	58.45± 9.81	209.91± 1.16[ab]	2.69± 0.36
9	40.90	49.97± 2.25[ab]	54.82± 12.27	182.22± 40.32[bcd]	3.45± 0.22

Different letters (a, b, c and d) indicate significant differences between groups ($P<0.05$).

Table 4

Physiochemical parameters of the water of experimental aquariums (in main experiments) (means ± S.D)

Factors	*Amount in aquarium*
Temperature (°C)	20±1.4
Dissolved oxygen ($mg\ L^{-1}$)	>6±0.7
pH	7.5±0.5
Hardness ($mg\ L^{-1}$)	300 - 410±0.0

In main experiments, light conditions were similar to L_{c50} (i.e. 9L: 15D).

Averages of total length and total weight of experimental fishes were 7.00±0.63 cm and 4.95±1.07 g.

Means of some growth indexes of 4.95 ± 1.07 g carp fry have been shown in Table 5.

Table 5

Comparison between means of some growth indexes and food conversion ratio in normal carp fry affected by different volumes of Halamid (main experiments)

Experimental groups	*Concentration of Chloramin T (mgL^{-1})*	*Percentage of increase in body weight (%/g)*	*Specific Growth Rate (g/d)*	*Food Conversion Ratio (g)*
1	0	249.1± 4.53	2.23± 0.02	0.67± 0.01
2	5	224.4± 24.96	2.09± 0.13	0.75± 0.08
3	10	232.6± 2.95	2.14± 0.01	0.71± 0.00
4	15	280.4± 25.79	2.34± 0.25	0.60± 0.12
5	25	200.4± 10.16	1.96± 0.06	0.84± 0.05

According to Table 5 and by using statistical analysis we found that there is no significant difference between percentages of increase in body weight, Specific Growth Rate and Food Conversion Ratio through experimental groups (P>0.05).

Percentages of increase in body weight of carp fry in main experiments have been shown in Figure 1.

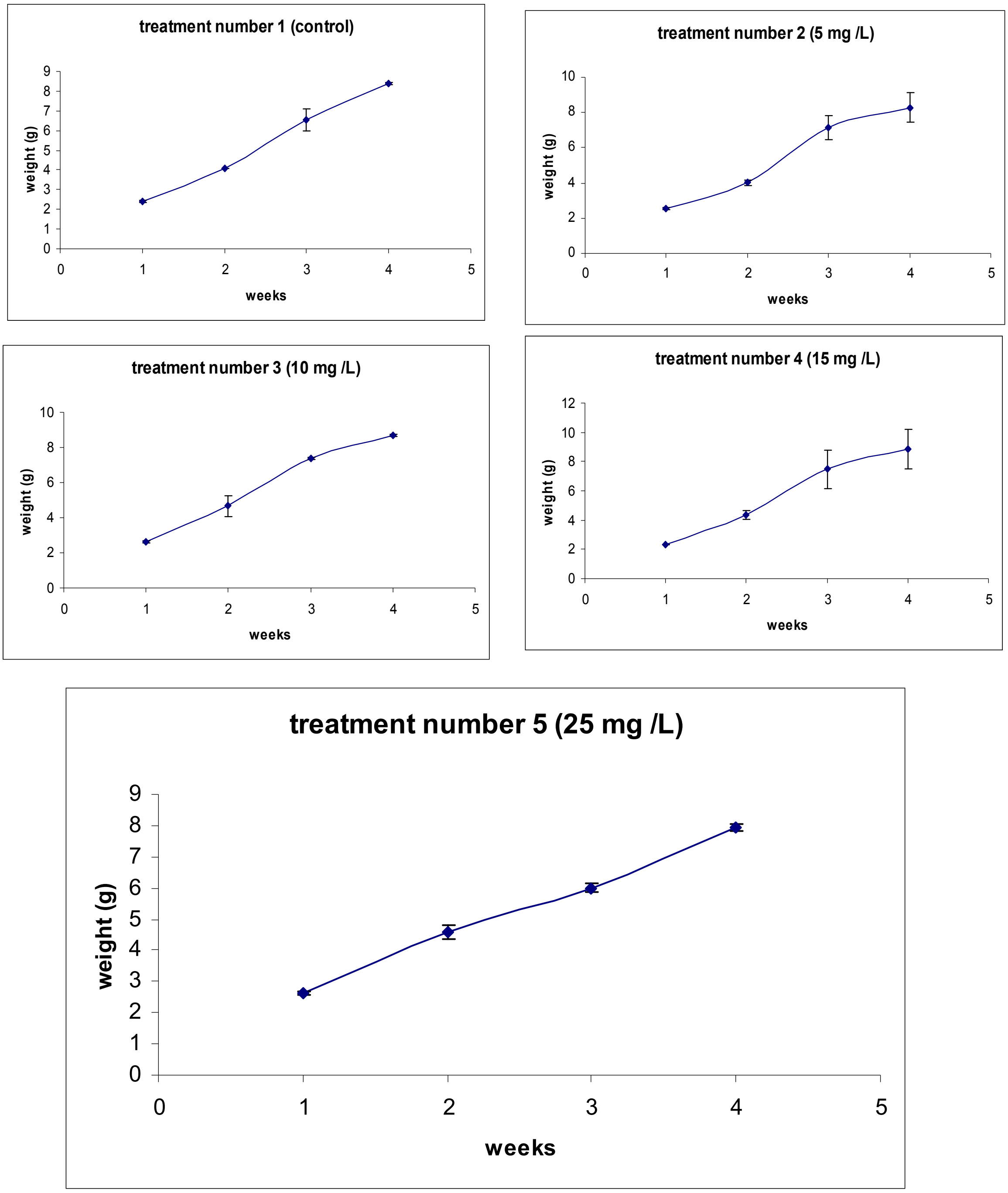

Figure 1. Percentages of increase in body weight (Average of total length and total weight were 7.00±0.63 cm and 4.95±1.07 g) of carp fry in different treatments.

Analysis of our results showed that at the end of the eighth week there was no significant difference between lengths and total weights of fishes which were exposed to different levels of Halamid (P>0.05). Percentages of haematocrit and biochemical analysis of serum in blood of 4.9 ±1.07 g carp fry in main experiments have been shown in Table 6.

Table 6

Comparison between means of percentages of haematocrit and amounts of glucose, cholesterol and total protein serums of carp fry in different volumes of Halamid (main experiment)

Experimental groups	*Concentration of Chloramin T (mg L^{-1})*	*Percentage of haematocrit (%)*	*Glucose (mg dL^{-1})*	*Cholesterol (mg dL^{-1})*	*Total protein (g dL^{-1})*
1	0	40.74± 2.17[b]	122.01± 2.27	148.10± 20.30[bc]	3.61± 0.45
2	5	53.11± 0.22[a]	146.82± 0.79	134.92± 17.59[c]	3.32± 0.24
3	10	57.34± 4.99[a]	99.54± 1.89	230.14± 45.33[b]	2.82± 0.06
4	15	43.58± 2.70[b]	108.39± 0.79	362.05± 24.38[a]	3.11± 0.56
5	25	51.52± 3.15[a]	115.14± 2.35	216.26± 29.77[b]	2.84± 0.08

Different letters (a, b and c) indicate significant differences between groups ($P<0.05$).

Results of Table 6 show that percentages of hematocrit in the controlled group and in the fourth experimental group had significant reduction ($P<0.05$) and this percentage in controlled group was lower than others. Furthermore, there was a significant difference between amounts of cholesterol of carp fry; so that the lowest cholesterol level was observed in treatment number 2 and the highest rate was observed in treatment number 4 ($P<0.05$). But there was not any significant difference between the glucose and total protein amounts of different groups ($P>0.05$).

During the experiment, both fish groups (controlled and treatment number 4) which were exposed to different levels of Halamid, demonstrated same nutritional behaviors. In addition, there was no respiratory stress effect such as rapid breathing, increased rates of gill cover movements or floating fishes on the surface of water. Besides, there was not any mortality in these groups during 8 weeks.

L_{C50} is a useful criterion for acute toxicity. But if those substances which are not toxic up to 96 hours, remain in water for longer periods, they may change to toxic substances (standard method) (Naji et al 2006).

Several factors affect on results of toxicity experiments on aquatic animals, including water features or biological characteristics of under experiment fish species. Hence, using standard methods to minimize external variables is essential while performing experiments of acute and chronic toxicity test. Therefore, it is important to be sure that all testing organisms are selected from healthy groups and are randomly distributed in the different groups (Shariati 2001).

Temperature, pH, hardness and oxygen of the water ponds, where carp fishes are bred in, are respectively 15-30, 6.5-8, 10-400 and more than 5 mg L^{-1} (Peighan & Mashaea 2005) and none of our treatments have exceeded theses factors. This means that if Halamid increases in any of our treatments, it will not have any harmful effect on physical and chemical factors of water; those factors which prevent survival of carp fishes in normal environmental conditions.

On the other hand, Serivastava & Saksana (1997) reported that water quality conditions, particularly factors such as temperature, dissolved oxygen, hardness and pH affects on absorption and toxicity.

At the end of L_{C50} experiment, major changes in blood biochemical factors of those carp fishes which were exposed to different ratios of Halamid included reduced amount of glucose ($P>0.05$) and increased amounts of haematocrit and cholesterol ($P<0.05$).

In a study by Soltani & Rostami (2001) on the effects of Diazinon on some biochemical blood indices of Russian acipenser (*Acipenser guldenstadti* Brandt & Ratzeburg, 1833), haematocrit and total protein in the experimental group were fewer than in controlled group, but levels of glucose were more ($P<0.05$). These scientists also expressed that the decrease of the protein of fishes' plasma will lead to liver failure in those fishes which are exposed to pesticides.

A significant decrease in the protein of plasma of those carp fishes which were exposed to Malathionon has been also reported (Khattak & Hafez 1996, Sancho et al 1997).

Contrary to our results, Luskova (2002) has reported that in serums of those carps which were exposed to acute toxicity of Diazinon, a significant reduction in protein concentration was observed ($P<0.05$).

In experiments which were done in 2006 in Guilan Fisheries Research Centre, Lc_{50} 96h of Diazinon toxin on *Rutilus frisii kutum* (Kamenskii, 1901) and silver carp (*Hypophthalmichthys molitrix* (Valenciennes, 1844)) were, respectively, 0.34 and 1.9 mg L^{-1} (Nasari Tajan 1996).

Fathollahi (1998) determined the amount of L_{C50} 24, 48, 72 and 96 hours of Undosefalon toxin in common carp respectively as 0.0061, 0.0029, 0.0016 and 0.0019 mg L^{-1}. By comparing his results with our findings we find that in 96h, carp resistance to Undosefalon is less than its resistance to Halamid.

Naji et al (2006) found that Lc_{50} chloride cobalt in 10 gram carp fry were 327 and 328 mg L^{-1}. In this study, amounts of MATC, NOEC and LOEC were respectively 100, 50 and 200 mg L^{-1}.

Naji et al (2007) found that amounts of Lc_{50} 96h of zinc sulfate in 120 gram Carp in 24, 48, 72 and 96 h were respectively 78, 64, 56 and 50 mg L^{-1}.

The investigation by Lam (1998) on the effects of zinc sulfate on Carp (5- 8 g) showed that amounts of LC_{50} in fishes with 5 to 8 grams weight, in 24 and 96 hours, was respectively 23 and 17 mg L^{-1}.

Many behaviors of those common carp fry which were exposed to Halamid (during this research) correspond with conditions observed after White fish (Gharae et al 2006) was exposed to mercuric chloride poison and conditions observed after other fishes were exposed to agricultural pesticides including Diazinon and other heavy metals (Shamoshaki et al 2008, Mirzae 2004, Barak & Mason 1990, Mance 1990).

The conclusion of Part 1 (L_{c50}) of this study is that Lethal Concentration, Lowest Observed Effect Concentration, No Observed Effect Concentration of Chloramin T (Halamid) in common carp fry show that Halamid is a suitable and inexpensive disinfectant with little lateral effects on carp fry and also it is useful for treating some diseases and minimizing the effects of the manipulation. Therefore, since the use of Malachite green has been forbidden all over the world and because of its negative lateral effects on the species environment, using Chloramin T for 25 to 15 mg L^{-1} (in mentioned hardness) in bath form can replace Malachite green use to control pathological microorganisms (bacteria, viruses, fungus and parasites).

Generally speaking, biochemical and hematological fluctuations in fish is the first discovered and measured response which is caused by environmental changes (Stegeman et al 1992) and biochemical characteristics can give important information about the innards of organisms (Masopust 2000).

During 56-day of keeping fishes in different experimental environments, major changes in biochemical factors of serums of experimental fishes which were exposed to different levels of Halamid included significant increase in the percentage of hematocrit in the controlled group and fourth experimental groups ($P<0.05$). In controlled group this percentage was lower than others. Besides, there was a significant difference between cholesterol amounts of carp fry, so that the lowest cholesterol level was observed in treatment 2 and the highest one was observed in treatment 4 ($P<0.05$). However among experimental groups there was not any significant difference between glucose and total protein amounts ($P>0.05$). It should be mentioned that the exact mechanism(s) which increase(s) and decrease(s) mentioned blood factors is (are) unknown. These changes probably are due to direct effect of Halamid on hematological tissues (kidney and spleen).

Biometric parameters indicate general health of fish and the quality of environmental water. Velisek et al (2009) studied biometric parameters of those common carps which were exposed to terbutryn. Similar to our results, they did not find any significant difference between biometric parameters of common carp after 28 days ($P>0.05$).

On the other hand, Dewey (1986) after 306 days of exposing the brook trout (*Salvelinus fontinalis* (Mitchill, 1814)) to Atrazine (120μg L^{-1}) reported decreases in weight and length of the fish ($P>0.05$). Furthermore, Davies et al (1994) after exposing *Galaxias maculatus* (Jenyns, 1842) to Atrazine observed a reduction in growth rate. These findings are inconsistent with our results; although in these studies exposing time, the active material and fish species were different from our research.

The increase in percentage of haematocrit mostly occurs in stressful situations which will increase the oxygen conveyance capacity and will provide more oxygen for major organs in a response to higher metabolic requirements (Rutten et al 1992). The comparison between the final length, final weight and the percentage of haematocrit will show that those two groups which show highest growths, have the lowest haematocrit. Hence it can be concluded that the fishes in lowest stress groups, showed more growth compared to other groups with higher stress ($P<0.05$).

Amini & Oryan (2001) studied on the effects of sodium chloride stress on the percentage of haematocrit in carp. According to this study, in stressful situations, haematocrit index was reported 13% to 45% for this species. Our results showed that using Halamid in several groups increase the percentage of haematocrit more than what they found.

Similar to our results and according to a research by Svobodova & Pecena (1988), carp fish after being exposed to acute toxicity of Atrazine shows a significant increase in the percentage of haematocrit ($P<0.05$). Peighan & Mashaea (2005) showed hematocrit reduction after exposing rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) to different levels of Malachite green ($P<0.05$).

Unlike our results, according to a research by Velisek et al (2009), various amounts of terbutryn did not have any significant effect on the percentage of haematocrit on carp fish ($P>0.05$).

Cholesterol is a necessary compound of the structure of the cell membrane. Cholesterol is measured to show the food status in animals. Increased concentrations of cholesterol in serum can be a result of damages to liver or kidney syndrome (Yamawaki et al 1986; Sancho et al 1997).

Yamawaki et al (1986) investigated the effects of gallium on common carp. This research showed that concentrations of cholesterol and glucose in those fishes which were exposed to high levels of gallium respectively increased and decreased ($P>0.05$). These changes in cholesterol are similar to what we found.

On the other hand Srivastava et al (1995) exposed catfish (*Heteropneustes fossilis* (Bloch, 1794)) to below acute and the fatal concentrations of Malachite green and investigated its effects on biochemical parameters of blood serum. Their results showed the decrease in cholesterol and the increase in total protein ($P>0.05$).

Concentrations of glucose in blood serum are regulated by complex interactions of some hormones such as glucagons and cortisol; although the environmental stress can increase values of glucose in plasma (Martin & Black 1998). On the other hand, changes in levels of glucose in serum can be due to malnutrition or an injured kidney (Jacobson & Keller 2001).

Mekkawy et al (1996) demonstrated increases in glucose levels in Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)) after being exposed to 3 mg L^{-1} Atrazine ($P<0.05$).

The most portion of serum protein synthesizes in the liver and it can be used as an indicator of liver dysfunction. The reduction of the total protein concentration is the obvious feature of many diseases and may occur due to liver disease, the absorption reduction or the loss of protein (Bernet et al 2001).

Fish reactions during eight weeks of being exposed to different levels of Halamid were similar to findings of Velisek et al (2009) who exposed carp to different levels of terbutryn.

On the other hand, Oropesa (2009) found that those carps which were exposed to Simazine for a long time show reactions such as rapid breathing, increased rates of gill cover movements and floating on the water surface.

Conclusion. In this investigation, after obtaining $L_{C10, 50, 90}$ (61.8, 40.90 and 27.10 mg L^{-1}), Lowest Observed Effect Concentration (27.10 mg L^{-1}), Maximum Allowable Toxicant Concentration (4.90 mg L^{-1}), No Observed Effect Concentration (11.28 mg L^{-1}) of Chloramin T (Halaimd) in carp fry, and also the effects of this substance on growth and blood parameters of this species have been evaluated. Since Halamid affects on stress adjustment and it is useful in treatment of some diseases and in minimizing the effects of manipulation and illnesses, 15 mg L^{-1} Chloramin T (Halaimd) is the proposed amount that minimize fish stress and furthermore has a positive effect on the growth of carp fry. According to this study:

- Absorption Range of Chloramin T is between 10/27 to 60 mg L^{-1};
- using this substance up to 25 mg L^{-1} did not have any negative effect on growth and biochemical parameters of blood and hematology of crap fry;
- using 15 mg L^{-1} Chloramin T during growth stage of carp fry can cause good growth of this species and also can affect on the control of harmful microorganisms (bacteria, viruses, fungi and parasites);
- therefore, according to mentioned results, this substance can be used as a better alternative material for malachite green (because of the obsolescence of this substance in aquacultural industry).

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