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ORIGINAL ARTICLE

Determination of the Lethal Concentration and biological effects of Trichlorophon Organophosphate pesticides on Goldfish (*Carassius auratus) (Linnaeus, 1758*)

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ABSTRACT
In this study, the effect of different levels of Trichlorophon toxin concentrations was evaluated In order to determine the lethal concentration LC50 within 96 hours on Goldfish that collected from Khuzestan province. Experiments according to O.E.C.D. protocol were done completely random and constant water quality; 24±1° C and pH= 8-8.5 in the three levels treatments of Trichlorophon toxin concentrations; 0.1, 0.01 and 0.001 and three repetition. Averages were compared by Duncan test in 0.05 significant levels. Results showed LC50 was 9.5 mg per liter at the end of the 96-hours test. In this experiment, symptoms were appeared in the tested fish such as curvature of the spine, body tremors, head and tail fin bruises and irregular swimming clinically. According to the results Trichlorophon is classified as toxic pesticides category for goldfish.
Keywords: *Trichlorophon, Pathology, Lethal concentration, Goldfish.*

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INTRODUCTION

There are goldfish in Iranian Haftsin tradition as an aquatic species. This aquatic animal is very similar to farmed hydrothermal fish in biological characteristics. Adding pesticides is very common in the world in order to elimination of agricultural or aqua cultural pests in hydrothermal aquaculture farms. Poisons and toxins are used as pesticide in agricultural lands and as growth Control of phytoplankton and zooplankton (source of hydrothermal fish feeding) in fish farms. Obviously, these fields drain naturally to downstream farms and where are implicated too, but also they are involved even entered to river and these pollutants can be transferred to other regions. Consumption of pesticides because of the development of agriculture and expansion of cultivated areas increased. Agricultural pesticides entered to aquatic ecosystems by various ways (Rainfall, runoff and drainage etc.) [1]. As a result, The residues of pesticides or their metabolites can affect unforeseen injuries on non-target organisms [2].This toxins damaged physiological and biochemical structure of fishes and also finally could through the food chain affect human indirectly (12). Trichlorophon -S (1, 2-dicarboethoxyethyl) O, O-dimethyl phosphorodithioate is one of the most widely used insecticides in Iran so that the level of this poison is measured more than standard in Namak-Abrod and Neka river [1]. Trichlorophon is an organophosphate poison and Leads disorder of nervous system through inhibition of Cholinesterase enzyme [3]. One of these toxins is Trichlorophon organophosphate insecticide. In this study, pathological effects of non-lethal doses of this insecticide were evaluated on vital organs such as liver and kidney. Pesticide different treatments 0.1, 0.01 and 0.001 mg per liter were examined on Organs of Goldfish at times 12, 24,36h.

MATERIALS AND METHODS

During this study, four aquariums were selected at 30 x 30 x 35 cm dimensions. All of the aquariums were washed and were disinfected by brine and tap water was used to supplying. The volume of water was 30 Liters in each aquarium. Water temperature was 20 ° C and pH was 7. These conditions were similar in 4 aquariums. The water of the aquariums without fish was stored for 24 hours in order to elimination of

chlorine. All of fishes were disinfected in 2% Salt water for 15 minutes, afterward fishes were added to the aquariums as four different treatments. 6 normal Goldfish with average size 8 cm were used in each aquarium. Oxygenation was performed for 8 hours per day by 4 outlet of air pump to all aquariums. The conditions were constant in order to adapting fishes to water for 12 hours. In these circumstances, the water was very clear and fish behavior was normal. After this period, three concentrations of Malathion poison were added to the aquariums with Syringe for diffusion and were mixed completely. The experiment lasted for 7 days. The treatments Included:

Aquarium Number (1) containing 30 L of water, 6 fishes and 0.1 mg per liter Trichlorophon poison.
Aquarium Number (2) containing 30 L of water, 6 fishes and 0.01 mg per liter Trichlorophon poison.
Aquarium Number (3) containing 30 L of water, 6 fishes and 0.001 mg per liter Trichlorophon poison.
Aquarium Number (4) containing 30 L of water and 6 fishes Without Trichlorophon poison. (Control)

Fishes from 1, 2 and 3 treatments were removed after 2, 4 and 6 days respectively. Blood samples were collected from caudal trunk and in smaller fish, from heart individually. Blood was collected and spread. Necropsy was carried to taking out the liver and the kidney by scalpel blade, forceps and scissors. At the end of this period, the water temperature was 23 ° C in all three groups and the control group. Water pH were 7.1, 7.2 7.1 and 7.03 in control group, aquarium (0.1), aquarium (0.01) and aquarium (0.001) respectively.

The specimens were fixed in formalin (10%) and formalin was poured 10 folds then tissues to jars. The samples were stored for 10 days for fixation completely.

RESULTS

Degeneration of renal cells and renal tubular cells were observed. LC50 studies are also performed on the organophosphate poison. The purpose of this study was evaluation of the harmful effects of Trichlorophon organophosphate Poison on target organs of this fish and similar farmed fish.

The signs of any group were certain and symptoms occurred at particular period.

1 - Renal histological changes

In group 1(0.1 mg per liter Trichlorophon poison treatment) fishes stored two days in aquariums, the following effects were seen:

Necrosis of epithelial cells of the urinary tract and increase of the renal hematopoietic tissue, disappearance of urinary tract, and dominant presence of Melanomacrophage cells (MMCS) with deposition of haemosiderin in anterior and posterior kidney were seen. (Fig 1)

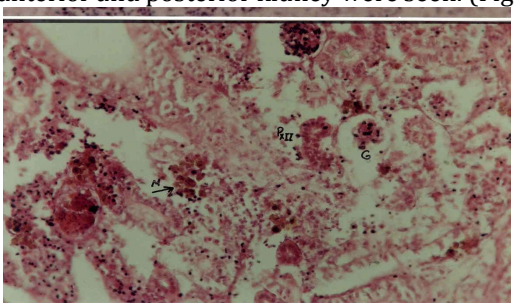

Fig1: Goldfish Kidney after expose to 0.1 mg Trichlorophon

In group 2(0.01 mg per liter Trichlorophon poison treatment), necrosis of urinary tract epithelial cells, proximal ducts I and II and dilation of the other ducts were evident. Inflammatory cells, eosinophilic secretions and the necrotic cells dug into the lumen of the urinary space were seen. Hyaline casts also were visible. Increase of hematopoietic tissues and disappearing of I and II proximal ducts brush border were seen. Vacuolated degeneration of renal tubules was evident even in collecting and mesonephric ducts. (Fig 2)

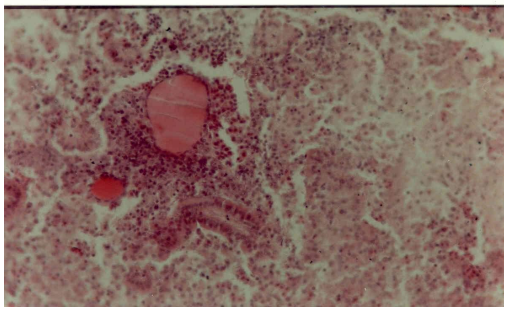

Fig2: Goldfish Kidney after expose to 0.01 mg Trichlorophon

In group 3(0.001 mg per liter Trichlorophon poison treatment), The presence of hyaline casts due to Protein reabsorption from the urinary tract wall that particles were finer than the mentioned prior particles . Despite glomerulus were normal, increase of hematopoietic tissue of posterior kidney and increase of urinary space (the distance between the inner and outer layer of Bowman's capsule) were seen. The presence of necrotic cells and inflammatory cells into the lumen of the urinary tract were prominent too (Fig 3).

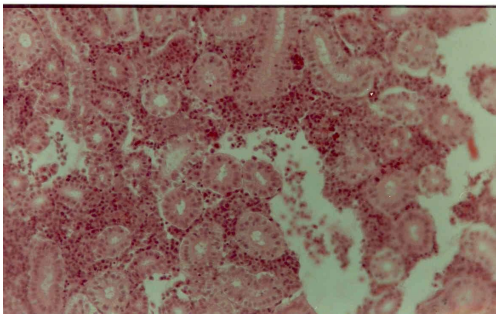

Fig3: Goldfish Kidney after expose to 0.001 mg Trichlorophon

Cloudy swelling and hypertrophy of urinary tubules cells and replacement of hematopoietic tissue instead of urinary tissue of the posterior kidney was seen.
The Hematopoietic cells and the urinary tubules in the control group were normal and regular completely.
2- The blood smear
Thrombocyte cells were seen, but there were severe hemolytic. (Figure 4)

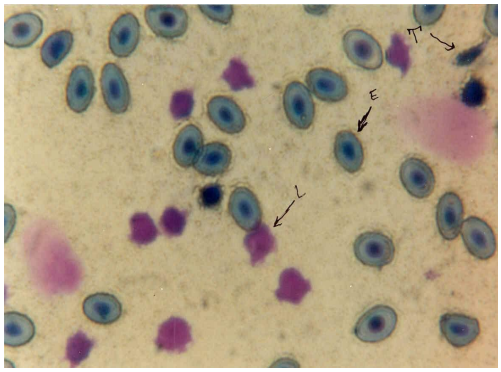

Fig4: Goldfish blood after expose to 0. 1 mg Trichlorophon

In the second group:
Erythrocytes were seen with monocytes (m). The presence of macrophages (M) in this concentration indicated blood circulation disorder and hemoglobin degradation. (Fig. 5)

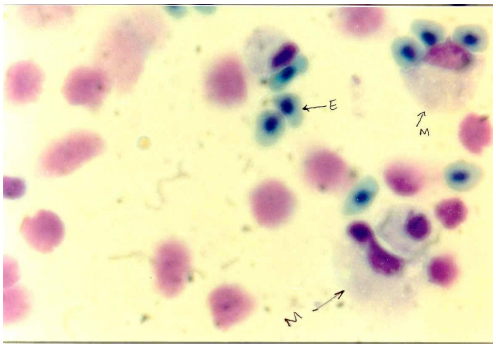

Fig5: Goldfish blood after expose to 0. 01 mg Trichlorophon

Erythrocytes were normal in the control group and other cells were less frequent. The aim of this study was determination of acceptable limits of Trichlorophon pesticide.

DISCUSSION

Aquatic ecosystems are one of the greatest parts of the natural environment and so a great deal of genetic diversity and biodiversity. High concentrations of usage pesticides in fish will reduce survival, growth and reproduction [4] and has obvious effects on fish [5].

Study of renal tissue showed, necrosis of epithelial cells of urinary tubules and destruction of urinary tubules like to [6]. Tubular degeneration was moderate and renal cells were involved moderate hemorrhage and pyknosis but Rahman (2002) study were more severe due to higher dose. Haemosiderin pigment in cytoplasm of renal cells is result of erythrolysis and hemoglobin catabolism; it should be considered that hematopoietic tissue in posterior part of fish kidney is very few. Hematopoietic tissue was increased in posterior kidney in 0.01 mg/l concentration because of demand more blood as a result hematopoiesis by anterior kidney. Similar findings were taken in hematology by mishra et, al (1983) who had used non-lethal concentration of Trichlorophon in different times. Some of the similar cases to present study were leucopenia and haemolysis [8]. Blood smear showed haemolysis can be as a result of erythrolysis and probably hemolytic toxic anemia that was accommodated to leukemia in0.1 mg/l concentration and increasing of macrophages in 0.01 mg/l concentration. Erythrolysis lead to decrease blood-oxygen transferring and intoxication [9]. Presence of polychromatocyte cells (immature red blood cells) were interpreted reason of compensatory anemia in intoxicated fish. Extension of industries lead to form this pollution in developing countries and our country, especially in south basin, different petrochemical contaminations are visible that one of the causes of mortality and different diseases in natural sources [10] [11]. Different plans instead of nowadays toxin that are advised by bio-environment protection organization can prevent fish mortality. These plans are based on evaluation of rivers pollution or design wastewater treatment plant for main country rivers and replacing some pesticides that have fewer intoxicity and fewer concentration of these pesticides have more potential for pecticiding.

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