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CHLORPYRIPHOS INTOXICATION ON TOXICITY, BEHAVIOUR AND BODY COMPOSITION OF Clarias batrachus

Dr. Abarna Roy

Department of Zoology Mayurakshi Gramin College Ranishwar Dumka, Jharkhad Pin-814148

Introduction

An insecticide is a pesticide used against insects in all developmental forms. They include ovicides and larvicides used against the eggs and larvae of insects respectively. Insecticides are used in agriculture, medicine, industry and the household. The use of insecticides is believed to be one of the major factors behind the increase in agricultural productivity in the 20th century. Nearly all insecticides have the potential to significantly alter ecosystems; many are toxic to humans; and others are concentrated in the food chain. It is necessary to balance agricultural needs with environmental and health issues when using insecticides.

. Chlorpyrifos is a toxic crystalline organophosphate insecticide that inhibits acetylcholinesterase and is used to control insect pests. Trade names include Dursban (home and garden uses), Empire and Lorsban (agricultural uses).

Pesticides that enters the aquatic organism often may not produce an immediate adverse effect such as mass mortality of a particular group of organism. But they may induce adverse effects in the long run. The variety of environmental factors, multiplicity of chemicals and their formulation may result in unpredictable interaction that can upset the stability of the ecosystem.(Mani and Konar, 1990)

Most of the pesticides applied for the pest control in agriculture and forestry enter the aquatic environment through various routes, polluting the aquatic ecosystem. The persistence of these toxic chemicals in aquatic environment may be dangerous for the survival of fish (Johnson, 1968; Saunders, 1969; Maedesley – Thomas, 1971). Pesticides reach our aquatic environment either by direct application on the water surface or by aerial drift or runoff or seepage from adjoining treated areas and create problems in fishery management. Analysis of samples taken in connection with several public health service investigations and national water quality basic programmes had shown presence of pesticides in soil run-off from treated areas (Doudoroff et al,1953).

Toxic effect of insecticides has been investigated by a number of investigators (Datta, 1980; Vardia and Durve, 1980; Thakur, *et al*, 1981; Singh and Srivastava, 1982; Hall, *et al*, 1982; Mahboob Basha, 1983; Kaur and Virk, 1983; Mishra and Thakur, 2006, Mishra et al, 2008).

Present investigation is an attempt to gather some

information about the toxicity of the Chlorpyrifos.LC_{50} value, behavioural changes and changes in the body composition after the exposure of Chlorpyrifos have been taken into consideration on a fresh water air-breathing fish *Clarias batrachus* (Linn.)

Materials and Methods

Live specimens of *Clarias batrachus* were procured from Dumka fish market and were acclimatized in laboratory before experimentation. The fishes were kept in big aquaria (50 gallon capacity). The animals were fed with chopped goat liver and earthworms. Care has been taken to keep the animals healthy and free from parasites.

In 96 hour, bioassays were conducted employing the technique of static test Doundroff *et al*., 1951; Thakur *et al*., 1981) in glass aquaria (60 x 30 x 30 cm) in the laboratory at ambient water temperature (29.0 – 32.9^{0}C) in the month of April– June 2006. The physicochemical characteristics of water were analyzed by APHA *et al*., (1980). These were water temperature 29.0 – 32.9^{0}C; pH 7.5; dissolved oxygen 6.8 mg/l; free CO_2 4.7 mg/l and HCO_3^- 297.0 mg/l.

Technical grade Chlorpyrifos (20% W/W EC) was used in present toxicity test. The fishes used were of same sex and body weight (male; 40-50 g). Preliminary test were carried out to estimate the LC_0 and LC_{100} values, followed by 96 hr exposure to 10 animals each group in 40 litres of solution in test concentration between 0.0375 to 2.4 ml/l of Chlorpyrifos. The mortality of

fish in each test concentration was recorded. The bioassays were repeated thrice, so that in all 30 animals (10x3) was exposed to each test concentration. The toxicity response data were calculated at confidence limits of 95% level and graphically represented on semi–logarithmic paper with test concentrations on the logarithmic scale and mortality percentage on arithmetic scale (Annes, 1975).

The subjective visual observations on the behavioural responses of the fish exposed to Chlorpyrifos were made. The number of aerial excursions and opercular movements were recorded in 0.009375, 0.01875, 0.0375, 0.0754 and 0.15 ml/l Chlorpyrifos exposed animals from 1 to 24 hr.

In second set of experimental condition 10 male fishes of about 50 – 55 g body weight were kept each in two aquaria. Group 'I' was treated as control, while group 'II' was subjected to longer exposure of effect of Chlorpyrifos for 15 days exposure at concentration 0.01875ml/l and effects on body weight, liver weight and hepato somatic index (HSI) and gonado-somatic index were determined.

The water content of liver and gonad was obtained from the loss of weight that occurred during drying at 55^{0}C. The 15 days exposure experiments were conducted in the laboratory in circular plastic pools (2×0.8m) and the Chlorpyrifos treated water of these pools were renewed at every 24 hrs as per recommendation of APHA, *et al*, (1975).

The hepato-somatic index (HIS) and gonado-somatic index (GSI) were calculated by the following formula:

$$HSI = \frac{\text{Liver weight}}{\text{Body weight}} \times 100$$

$$GSI = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100$$

Students't' test was applied to evaluate the significance of difference at 5% level.

Observation

The results of acute toxicity test have been shown in Table-1, 2, 3 and the toxicity response curves have been represented in Plates I and II. On the basis of interpolation of the data, the LC_{100} and LC_0 were calculated to be 1.2 ml/l and 0.01875 ml/l. LC_{50} value of 0.15 ml/l is much close to the observed values (Plates I and II).

When fishes were exposed to higher concentrations of Chlorpyrifos, hyper excitability, increased aerial excursions and opercular movements were observed as immediate response of fish towards the toxicant. The Fishes showed restlessness and tried to jump out of aquarium. Fishes were often observed swimming with jerky movements. After some times fishes settled to the bottom of the aquarium but was in restless condition.

In higher concentrations of Chlorpyrifos fishes showed red wound patches on the skin surface at different places of the body. Marked discolouration of the skin after Chlorpyrifos exposure has been observed. Similar red patches were also observed on the gill surface and at the bases of dorsal,pectoral,pelvic and caudal fins of the Chlorpyrifos exposed *Clarias batrachus*. Swelling of base of fins was also recorded in exposed fishes. The terminal phase was characterized by agitated movements, tremors, convulsions and loss of balance. Shedding of skin of body surface and bases of fins also occurred. This caused water of the aquarium to become dirty. Before death fishes became senseless and finally settled to the bottom of water in the aquaria.

The visual respiratory response and rate of surfacing of fish after exposure to various concentrations of Chlorpyrifos has been shown in Table-4 and graphically represented in Plate-III. The 24 h mean values of opercular movement and rate of surfacing increases significantly with increasing concentrations of Chlorpyrifos At higher concentration, i.e., 0.15 ml/l the fishes showed maximum opercular movements and increased rate of surfacing to the extent that the animal tried to jump out of the surface of water.

The feeding rates decreased with the increasing concentration of Chlorpyrifos. This visual observation was further supported from the analysis of the gut content. The gross food conversion rate could not be worked out since the discarded feed left in the aquarium was not accounted. The growth rate of tagged animals in 15 days decreased to 18.5 per cent against control.

The effect of 15 days exposure of Chlorpyrifos at a concentration of 0.15ml/l has been shown in Table-5 and represented by bar histograms in Plate-IV. The body weight has reduced from (49±3.4641 to 39.9 ±3.3813g). There is significant decrease (18.5 %) in the body weight of treated fishes in comparison to controls. The liver weight have reduced to (0.83±0.2162 to 0.6 ± 0.2447) and gonad weight (6.46±0.3204 to 3.95±0.3027) have reduced in comparison to controls respectively. The liver water and gonadal (testis) water has significantly increased (P>0.001 respectively). There is 54.48% and 49.12% decrease in hepato-somatic index and gonado-somatic index in 15 days exposed fishes to Chlorpyrifos in comparison to controls.

Discussion

Chlorpyrifos (O,O-diethyl O-(3,5, 6-trichloro-2-pyridinyl)- phosphorothioate) is an organophosphorus (OP) insecticide used for controlling insect pests. The contamination of water by pesticides can have deleterious effects on organisms. The nature of the effects varies but can include structural and functional modification at both the cellular and sub-cellular levels in a variety of organisms (Verma,et al,1981).

A critical evaluation of the previous literature indicates that the effect of many pesticides and synthetic fertilizers on the freshwater fishes have been studied extensively and there is comparatively less work on the effect of Chlorpyrifos on this group of fishes , although these compounds are commonly used in

agricultural fields in this area. Toxicity is a relative property of a chemical which refers to the potential to have a harmful effect on living organism (Rand and Petrocelli, 1985).

Temperature conditions in toxicity tests are important parameters to be considered (Cope, 1966; Walker, *et al*, 1964; Henderson, et al, 1959 ;Thakur, *et al*, 1981; Thakur and Pandey, 1988 ;Sah, *et al*,2007. Basak and Konar, (1977) reported 168 hours Lc_{50} of BHC for *Heteropneustes fossilis* to be 7.58 ppm at 20-35^{0}C water temperature having 7.0 pH. Water temperature is one of the most important factors in the environments of aquatic organism and plays a vital role in determining their distribution, growth, reproduction, metabolism and behaviour Sah, *et al*, 2007.

The hardness of water, alkalinity and pH have no major effect on the toxicity to fish of chlorinated hydrocarbons (Henderson, *et al,* 1960). Gamaxene is reported to be more toxic to *Channa punctatus* and *Heteropneustes fossilis* in comparison to Anthio – 25, Dimercon and Sevin (Chakravarti and Chaurasia, 1981); and on common carp, *Cyprinus Carpio* (Kaur and Virk, 1983).Cypermethrin has been found to be more toxic in *Channa punctatus (*Mishra and Thakur,2006).

Animals are sensitive to chemical signals at low concentration and they may rely on this sensory input to control their attitude and behaviour (Todd, *et al*, 1967). There is increasing realization that the effect of pesticides on the reactions of fish other than the easily observable mortality

effects, must be taken into account in evaluating the complete ecological impact of a contaminating substance.

Visual observations on behavioural responses of *C. batrachus* to lethal and sub lethal concentrations of Chlorpyrifos are in agreement with those of *Cyprinus carpio* (Datta, 1980),

Cyprinus carpio* , *Poecilia recticulata* (Jivasek, 1981), *Barbus

Stigmata* (Manoharan and Subbiah, 1982) and *Atherinops affinis,

Rhaecochilus vacca, Oxyjulis Californica, Chromis punctipinnis,

***Girella nigricanus, Lythrypnus dalli, Hypsoblenius jenkinsi* (Hope, Stoffel and Zebra, 1983) exposed to different organochlorine pesticides. The report of lindane poisoning on brown trout, *Salmo trutta* L. by Drewett and Abel (1983) is in agreement with the findings on *C. batrachus* exposed to Chlorpyrifos. The reaction of fish can be in general characterized by erratic marked wound patches, discoloration and depigmentation of skin, ataxia and intermittent paralysis. Similar behavioural response to Zinc poisoning has also been reported in *Channa punctatus* by Khangarot (1982), malathion poisoning on *Hetropneustes fossilis* by Chaudhary, Pandey and Dubey (1981), *Notopterus notopterus* by**

Verma, Tonk and Kumar (1983),urea exposure on *C. punctatatus by* Sah, *et al*,2007.

Although there have been many reports of pathological lesions caused by pollutant in fish, there have been few systemic descriptions of the toxic syndromes associated with exposure to pollutants. Such descriptions are clearly desirable as an aid to understanding the

mechanism of action of pollutants, and could be diagnostic aids in the investigation of fish mortalities where the accidental or negligent discharge of pollutants is suspected. The symtematology, leading to death produced by the exposure to Chlorpyrifos in the present study was indicative of the general toxic effects produced by most of the organochlorine insecticides. Drewett and Abel (1983) reported effect of lindane poisoning on *Salmo trutta*, at death the gill filaments were a bright red colour, indistinguishable from control fish. This colour gradually faded until at approximately 2h after death the gills were white this occurred in all lindane – exposed fish irrespective of the poison concentration. Gills of control fish faded slightly within the fish 2h after death to a pink colour, and did not turn while after rigor mortis had passed and decomposition begun after 20 h.

Death of fish exposed to lethal levels of Zinc involves tissue hypoxia (Skidmore, 1970; Burton, *et al*, 1972) and it may be therefore that fish killed by pollutants may show pathological sign of hypoxia in addition to signs specifically associated with the poison. Similarly of the signs of lindane poisoning and of hypoxia in *Salmo trutta* suggest that death from lindane poisoning may be associated with tissue hypoxia (Drewett and Abel, 1983). Physiological and biochemical studies on fish exposed to lethal levels of Zinc (Skidmore, 1970; Burton *et al*., 1972) indicate that tissue hypoxia is a result of Zinc poisoning and probable immediate cause of death, since zinc is not a potent internal poison (Skidmore, 1970). The convulsion and ataxia shown by Chlorpyrifos poisoned fish suggest that Chlorpyrifos has a neurotoxin action in fish. If tissue hypoxia occurs as a result of Chlorpyrifos

poisoning, it is likely to be due to loss of co-ordination in the cardiovascular and / or respiratory systems. Similar observations have been made by O' Brien (1967) in insects and mammals with lindane poisoning.

It is generally recognized that fish respond to toxic chemicals by increased opercular movements (Belding, 1929). The stickleback breathes normally about 120 times per minute at 17^{0}C. When heavy metal salts (Copper sulphate, 64 ppm and lead nitrate 500 ppm) are put into the apparatus the rate of opercular movement and rate of Oxygen consumption rise. This is due to increased activity, as the fish senses the unfavourable changes in its environment and struggles. As the toxic process advances the respiratory movements become more and more rapid, more regular and of increased amplitude. Despite the animal efforts to maintain its oxygen supply, the oxygen consumption falls, returns to normal and became subnormal. After periods of struggling and rest, and minor fluctuation in the opercular movement, the fish sooner or later becomes exhausted. When the rate of oxygen consumption sinks to about 20 per cent of normal the opercular movement begins a precipitous descent and fish dies (Jones, 1948; 1964). The effect of an insecticide Chlorpyrifos on *Clarias batrachus* shows increased opercular movement .A number of published works are in agreement with the present finding (Jones, 1948, 1964; Annes, 1975; Kumar, Pant and Khanna, 1979; Srivastava and Srivastava, 1979; Datta, 1980; Khangarat, 1982; Arunachalam and palanichamy, 1982 ; Mishra and Thakur,2006). Chaudhary, Pandey and Dubey (1981) observed irregular opercular frequency in *Hetropneustes fossilis* exposed to

malathion. In *Hetropneustes fossilis,* the opercular movement increased enormously for 1st few minutes, but the average opercular frequency has decreased in 24 h time. They showed high correlation of coefficient against concentration. Nagendran and Shakuntala (1979) observed that under the exposure of sub lethal concentrations of sodium

pentachlorophenate, *Puntius ticto* exhibited significant increase in the opercular / surfacing activity. Sah, *et al*,2007, showed effect of urea on body composition and behaviour of *C .punctatus* and observed decline in opercular movement and aerial excursion.

Basak and Konar (1977) observed deformed taste buds and reduced feeding rate in *Cyperinus Carpio* exposed to

DDT and increased growth rate in *Tilapia mossambica* by 28% in DDT treated ponds. Holmberg, *et al*, (1972) recorded considerable loss of body weight in *Anguilla anguilla* exposed to pentachlorophenol found reduced feeding, and growth rate in *Heteropheutes fossilis* exposed to malathion. In the present study on *Clarias batrachus* exposed to Chlorpyrifos, a reduction in body weight as well as in feeding rate has been observed. Similar results have been obtained in fingerlings of *Cyprinus carpio* exposed to endrin (Datta, 1980); *Poecilia sphenops* (black molly) exposed to chloroform, tetra – chloroethylene and trichloroethylene (Lockie, Schecter and Christain, 1983). Manoharan and Subbiah (1982) showed that sub lethal concentrations of endosulfan causes marked reduction in feeding rate from *Barbus stigma*.

Verma, Tonk and Kumar (1983) reported that Thiotox (T), and organochlorine and Malathion (M), organophosphate insecticides exposed *Notopteus notopteus* consume lesser amount of food compared to unexposed fishes. The rainbow trout, *Salmo gairdnei* after prolonged exposure of hexavalent chromium showed no growth retardation (Pulte, Galilean and Strik, 1982). Food intake of *Macropodus cupanus* reared in different concentrations of Carbaryl did not vary significantly while growth decreased with increased concentration of Carbaryl possibly due to increased expenditure of energy on metabolism (Arunachalam and Palanichamy, 1982). Thus it appears quite clearly that reduction of body weight and feeding rate occurs after toxicant specific reaction (Thakur, 2007).

The body composition of fish has been reported in relation to size, age (Lovern, 1938), sex, locality (Zinevici, 1970), nature of food (Hornell and Nayudu, 1924) and season (Philips, 1969) but information related to the effect of pollutants on body composition are scanty. The moisture content of freshwater fishes has been reported mostly between 70-80% although values as low as 53.7% in *Hilsa ilisha* and high as 88.8% in *Cyprinus Carpio* are not uncommon (Anonym, 1962).

When *Clarias batrachus* are subjected to 15 days exposure of 0.15 ml/l of Chlorpyrifos there is a loss in body weight, liver weight gonad weight and hepato-somatic index and gonadosomatic index, while water content of body ,liver and gonad in exposed animals have increased significantly after Chlorpyrifos treatment. (Choudhary, Pandey and

Dubey,1981), demonstrated that the water and lipid contents of the whole body and ovary decreased compared to control. The liver water increased from (75.492± .082 to 86.5±1.9) with the increasing concentration of malathion in *Heteropneustes fossilis*. Verma, Tonk and Kumar (1983)reported increased HIS and decreased water content of liver

of *Notopterus notopterus* exposed to thilox (T), an organochlorine, Malathion (M), an organophosphate and M/T and T/M combinations. A significant decrease between 27 % and 43% in the liver size and liver somatic index of Flounders were observed as a result of cadmium exposure (Larsson, *et al*, 1976). The effect was not due to a drop in the water content of the liver, as this parameter was unchanged. It has also been shown in mammalian experiments that cadmium induces various changes in liver; from at least activity to certain lever enzymes to serves liver cirrhosis (Nilson, 1970). However, cadmium induced effects on the liver have probably been over looked in the past (Nordberg, 1974).

The decreased liver weight of Chlorpyrifos, exposed fishes as observed in present investigations is similar to those reported by various authors (Larsson, *et al*, 1976; Nilson, 1970; Nordberg, 1974). The decrease in liver weight is not due to decrease in water content but possibly because of decrease in protein, fat and other solid materials. Increase in liver water suggests very strongly that there is degradation of protein and fat of the liver. It has been reported that an animal may loss practically all of its fat and half of its protein and live but loss of only 19% of its water causes death

(Maynard and Loosli, 1962). Similar observation has been made by Thakur, 1987.

The LC_{50} value for this fish has been calculated to be low. Generally Chlorpyrifos, applied at the rate of 50 lb/A in the field. The Chlorpyrifos is found to be much toxic at very low concentration in present investigation on *Clarias batrachus*. As LC_{50} of Chlorpyrifos is determined to be very low i.e. 0.15ml/l in

***Clarias batrachus*. Thus there is every chance of aquatic contamination by this pesticide. In previous studies with much hazardous and persistent chemical, like Organochlorines such as DDT, Lindane, BHC etc. and Organophosphate insecticides accumulation in the body, has been reported by many workers in the past.**

The Chlorpyrifos is highly toxic but not persistent chemical. It disintegrates at faster rate. Due to this property it is considered to be friendlier by the farmers in comparison to other pesticides. Further investigations are to be encouraged to finally establish Chlorpyrifos contamination to be declared safe for various water bodies.

Summary

Investigations were undertaken to record the effect of Chlorpyrifos, an insecticide on toxicity, behaviour and body composition of an air-breathing fish, *Clarias batrachus* (Linn.). The 96 hours toxicity limit (TLm) value for this fish has been calculated to be 0.15 ml/l.

The behavioural response of the fish towards toxicant was grossly dependent on concentration and length of exposure. Increased aerial excursions and opercular movement was observed as immediate response of fish towards the toxicant.

Liver weight, Hepato somatic index (HSI), gonad weight, gonado-somatic index (GSI) decreased with increasing concentration of Chlorpyrifos. There was marked increase in liver water (16.26%) and gonad (19.80%) water in animals exposed Chlorpyrifos for 15 days. Decreased feeding rate, growth rate and hydration of liver and gonad indicate the toxic effect of

Chlorpyrifos.

When fishes were exposed to higher concentrations of Chlorpyrifos, hyper excitability, increased aerial excursions and opercular movements were observed as immediate response of fish towards the toxicant. The Fishes showed restlessness and tried to jump out of aquarium. Fishes were often observed swimming with jerky movements. After some

times fishes settled to the bottom of the aquarium but was in restless condition.

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