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RESEARCH ARTICLE

TOXICITY OF CHLORPYRIFOS TECHNICAL GRADE AND 20% EC TO THE FRESHWATER FISH, *CHANNA PUNCTATA* (BLOCH)

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Abstract

The toxicity studies of chlorpyrifos technical grade (TG) and 20% EC are conducted in the prevailing laboratory conditions of the fish Channa punctata in vivo to determine the median lethal concentrations i.e., LC50 values for 24, 48, 72 and 96 hrs duration by employing both static renewal and continuous flowthrough systems. The most preferred and palatable fish and mostly used pesticide the organophosphate are selected for study. The static renewal values are 6.02 µg/L, 5.82 µg/L, 5.62 µg/L, 5.38 µg/L for technical grade and for 20% EC 3.01 µg/L, 2.71 µg/L, 2.61 µg/L, and 2.51 µg/L for 24, 48, 72 and 96 hrs respectively. Similarly, for the continuous flow through system (CFTS) the values are 3.11 µg/L, 2.87 µg/L, 2.81 µg/L and 1.61 µg/L for technical grade and for 20% EC 1.91 µg/L, 1.81 µg/L, 1.61 µg/L and 1.41 µg/L respectively for 24, 48, 72 and 96 hrs. The toxicity is the concentration and duration dependent and the LC50 values in both methods decreased as well as concentrations also. The static renewal values are higher to CFTS and 20% EC is sufficiently toxic, which had synergistic effect on target and tested organism. The values are useful for data base and also in prescribing the permissible limits.

Introduction:-

In 2019 Macnamara [1] the UN's Global Environment Outlook (GEO) called for the usage of pesticides that should be reduced substantially, so that the pollution too, should be minimised, and can cut to sizeable extent. The aim of the concept was not only the food production to the man but also for the entire biodiversity conservation and preservation which is also the criteria for consideration. As the environment of air, fresh water and marine water got defilament due to pollution and due to globalization, industrialization, 'sustainable agriculture' and sustainable living can be possible, to a certain extent by limited pesticide use, reduction in the loss of food and waste of it.

Tang *et al*. (2021) [2] cautioned globally, that higher the risk score, the higher the probability for a non-target species to experience an effect, which might be as severe as death. Statistically, the futuristic outlook by them of their thinking preserved that South Africa, China, India, Australia and Argentina as regions of high risk zones of

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pesticidal pollution. The surface water are richly contaminated and in philosophical terms mentioned that regions were considered to be high risk of residues of at least one of the pesticide ingredients that were estimated to be at least 1,000 times greater than concentrations that would produce no effect.

Anamika Srivastava *et al*. (2018) [3] and Suchela Yadav and Subroto Dutta (2019) [4] reported on the pesticidal pollution of water, wherein these chemicals takes a loins share in contamination and also the patterns of pesticide consumption in a regions of Rajasthan who projected the Indian scenario, where such residues cause damage not only to water but also the non-target organisms fish, which account sufficiently more in number in the natural waters, forming a connecting link of food chain to the terrestrial environment.

Liana Christiana Soare *et al.* (2019) [5]. Ullah and Zorriezahra (2015) [6], Murty *et al*. (2013) [7], Sunanda (2016) [8], Deb and Das (2013) [9] in their review articles opined that fish is a good model to toxicity studies while reporting that the LC_{50} values for different fish as well as to chlorpyrifos, the organophosphate.

Toxicity is the first parameter to be assessed for toxicant effect, while doing so the determination of both acute and chronic toxic evaluation. In the ambient when the nektonic poikilothermic fish, had the impact of the chemicals being entered through gills of them along with water apart from mouth.

To assess the toxicant action for the mortality rate is taken into account to arrive percent kills for a species concentration exposure in the laboratory conditions. Doudroff *et al*. (1953) [10], first employed the toxicity tests later, committee on methods of toxicity tests with fish invertebrates and Amphibians, Anon (1975) [11] given by environmental protection agency (EPA). Finally, American Public Health, American Water Works Association, Water Environmental Federation, Washington DC, USA, in the years 1998, 2005 and 2012 drafted certain guidelines and recommended four different methods of toxicity, in which in the present study, static renewal and CFTS methods are only applied to determine the toxicity values of acute nature..

Material and Methods:-

The fresh water fish *Channa punctata* of size 10-12 cm in length and 10-12 gms wt are brought from local fish market and the toxicant of technical grade is from Hindustan Agro Chemicals, Ahmadabad, India and 20% EC is purchased from the local market.

Initially they are acclimatized to the laboratory conditions in the tubs of the plastic material. The water is unchlorinated ground water and were fed for 15 days at the room temperature of 28+2^{o}C. The physico-chemical parameters the water used for conducting experiments are:

Physical & Chemical properties of water used for the present experiments are (in mg/L): Turbidity–8 silica units. Electrical conductivity at 28^{o}C-8.16 Micro ohms./cm, pH at 28^{o}C-8.2. Alkalinity**:** Phenolphthalein-Nil, Methyl orange as $CaCO_3$-472, Total hardness-320, Calcium Handness-80, Magnesium Hardness-40, Nitrite nitrogen (as N)- Nil, Sulphate (As SO_4) – Trace, Chloride (as Cl^-)-40, Fluoride (as F^-) – I.S. Iron (as Fe)- Nil, Dissolved Oxygen – 8-10 ppm, Temperature - 28±2^{o}C.

While experimenting in the toxicity durations of 24, 48, 72 and 96 hrs, the fish were not fed. All the precautions that were there as recommendations of APHA, 1998, 2005 and 2012 [12,13,14] followed during the entire toxicity evaluation procedure. During such period, they were fed with oil cake made of groundnut and also with bran of rice.

All the precautions laid down by American Public Health, 1998, 2005 & 2012 [12,13,14] for conducting fish toxicity test procedures are only followed. The pilot experiments are first conducted to choose the concentrations to know the toxicity range in terms of effective concentration. In such exposure of the toxicant 10 fish are taken for each test and they are introduced in toxicant glass chambers with a capacity of 10 litres in static renewal tests. For continuous flowthrough system, reservoirs of 60 litres capacity are used and the test water is let into test containers at a rate of 4 litres per hour using poly-ehylene drip sets with regulators and for every 12 hours fresh test solutions were prepared in the reservoirs.

The two types of experiments are conducted to determine the toxicity of chlorpyrifos and 20% EC also in various concentrations to determine the fish deaths during experimentation. The data on the fish deaths i.e., the mortality rate of fish was recorded during the experimental period. The dead fish are removed immediately. The toxic tests are conducted to choose the mortality that range from 10% to 90% for 24, 48, 72 and 96 hours in both static renewal and continuous flowthrough system for both the toxicants.

Finney's probit analysis (Finney, 1971) [15] as recorded by Roberts and Boyce (1972) [16] was followed to calculate the LC_{50} values. The respective probit values were taken from Table-IX of Fisher and Yates (1938) [17]. For the determination of the 95% confidence limits, LC_{50} values and a normal variant of 1.96 were taken into consideration.

The data was subjected subjected to the following statistical equations for arriving LC_{50} values.

$$\text{Log } LC_{50} = \frac{\text{Log } A + 50 - a}{b - a \text{ Log}^2}$$

Where:

A : Concentration of pesticide at 50% Mortality.
B : Concentration of pesticide at 50% mortality

a = Per cent kill just below 50% mortality
b = Per cent kill just above 50% mortality

Regression lines of probit against logarithmic transformations of concentrations were made Slope function (S) and confidential limits (Upper and Lower) of the regression line with Chi-square for (UNEP/FAO/IAEA, 1987) [18] were calculated as follows:

$$S = \frac{\frac{LC_{84}}{LC_{50}} + \frac{LC_{50}}{LC_{16}}}{2}$$

$$F = \text{antilog } \frac{(277 \log S)}{\pi N} = S^{2.77} / \sqrt{N}$$

Where N is the number of animals tested whose expected effects are between 16 and 84% mortality.

Upper confidence limit = LC_{50}X f
Lower confidence limit = LC_{50} / f

Further, the data is also processed by the Probit analysis and the computer generated output is taken which had given not only the LC_{50} values, upper and lower limits of fiducial intervals, the regression equation, slope and Rf values (Finney, 1952) [19] and found that it is a good fit.

Results:-

The LC_{50} values for both the toxicants to the fish in static renewal test and continuous flow through system (CFTS) along with upper and lower limits of regression equation are given in the table 1, 2, 3 and 4 for 24, 48, 72 and 96 hrs respectively.

The regression equation along with probit value and percentage mortality is given as figures as graphical representation for 24, 48, 72 and 96 hrs for both the technical grade and 20% EC are appended as Figs.1-16.

For each exposure set of a test for 24 hrs, 48, 72, and 96 hrs five concentrations are selected. In testing the experiment of toxicity values, the sensitivity range of each ml containing the toxicant concentration differ for technical grade as 0.2 ml wherein for 20% EC it is 0.1 ml for commercial formulation the sensitivity of 0.1 ml containing 0.1 µg/L is making the fish to have an cumulative effect.

The static renewal values are higher to continuous flow through system as in the former dissolved oxygen document and accumulation of metabolites such as CO_2 and excretory wastes of ammonia. In CFTS, the conditions are better than to static renewal tests.

The values are concentration dependent as well as duration. As the concentration increases the percent kills are more for both the toxicants and the values decrease as the duration increase. Hence, the values as per table 1, 2, 3 and 4 decreased as the time progress from 24, 48, 72 and 96 hrs for both the toxicants.

Discussion:-

The toxicity for any type of the toxicant, even the pesticides it is species specific. The chlorpyrifos in no exception, as per the review articles of Sunanda *et al*. (2016) and Deb and Das (2013).

The acute toxicity hazard of the insecticide active ingredients to fresh water fish made it clear that chlorpyrifos, commercial formulations such as Lorsban NT, Lorshban 50W, pyrifos 15g and Pyrinex 480 EC, cautioned that it is toxic (EPA, 2009) [20].

The species variation of the toxicity in terms of $\mu g/L^{-1}$ (ppm) prescribed as tolerance for complete aquatic organism algae, amphibian, crustacean. Fish and insects showed that it is species specific and for fish it ranged as 0.53 $\mu g/L^{-1}$ minimum and a maximum >806 $\mu g/L^{-1}$ by static, static renewal and flow through test for chlorpyrifos and fish sensitivity is also.

The toxic action of the organosphates in general neurotoxic, as per the report of Natalia *et al*. (2019) [21]. The toxic action of the fish due to neurotoxic biomarker, renders the fish, more inhibition of the enzyme, Acetyle cholinesterase of the brain. The enzymes of such are mentioned to be polymorphic and action of it is more in brain that causes oxidative stress and release of the other enzymes such as antoxidant. In such situations, the reactive oxygen species production rate got enhanced, defense system being disturbed as an hypothesis, to be assessed. But however, an increase of GST activity and such things happening internally in the cells/tissues had impact on the physiological/biochemical harmony as homeostatis mechanism of operation that failed rendering to suffer. The muscle, cholinesterase activity is not on the part with brain and is less, so no movement of the fish, if it is going to be preyed upon by others, it was more likely to happen. But if the fish itself is carnivorous as in the present studied one, due to lack of muscle activity it may not feed probably and as such lack of feeding render the fish to suffer more hence might be the present organophosphate tested, chlorpyrifos had toxic action.

Tivany Edwin *et al*. (2019) [22] reported the toxic values of two fish, *Oreochromis niloticus* and *Cyprinus carpio*, exposed to chlorpyrifos. The values they reported by the static renewal, and the sizes of the two fish were different 0.19 µg/L for first and 0.07 µg/L for the second fish as sublethal concentration. The pathological lesions of the vital organs as in the present study which made them got degenerated as a consequence the fish showed toxic action rendering the fish to succumb the lethal action as ‘death’.

Arumugam Stalin *et al*. (2019) [23], considering the determined toxicity value as 5 ppm in the fish *Channa punctata*, where the size of the fish is more and the method of toxicity determination was only static which in no way comparable to the present study, opined, due to histopathological lesions of vital organs toxic action was resulted which even in the present study, reiterate the concludive findings.

Zahan *et al*. (2019) [24] reported LC_{50} values for chlorpyrifos, to the fish, *Heteropneusteus fossils*, *Channa punctata*, *Anahas testudaneus and Batasio tengana* using 20% EC by static method. The 96 hrs LC_{50} values were 23.1 µg/L, 20.3 µg/L, 16.6 µg/L and 13.9 µg/L respectively for cat fish, snake head fish, climbing perch and tengra. The snake head fish size was 12.88±0.98 cm and the present study one less is size and a comparative study of static renewal and CFTS. The values cannot have any comparison, but because the pH is 6.64 to 6.80 and temperature range 17-18±0.5^{o}C in their report of study whereas in the present study the pH and temperature are higher to their study. Behavioural attributes which are the indices of the toxicity were also mentioned.

Mohammad Ismail *et al*. (2018) [25] in the fish *Labeo rohita* exposed to chlorpyrifos, 24, 48, 72 and 96 hrs toxicity values, that were reported but the cause of the toxic action was due to haematological impediments as well as, the fish erythrocytic nucleus showed breaks (MN induction) that all the resultant, which even in present study, true in its effect. The size of the fish, methodology of the test to determine the toxicity evaluation and the very feeding habits were totally different. All these factors, showed variation of which toxic action by the constituent blood cells changed which is the one, that can be considered.

Revathi and Krishna Murthy (2018) [26], using the present studied toxicant to the fish *Channa striatus* of (size 10-12 cm and weight 50-100 gms) reported, the toxicity value for 24 hrs as 0.18 ppm by employing static test. The reason of the toxicity which in them opinion was due to proteolysis in the gill, liver and muscle and the fish due to stress after exhausting the carbohydrate source to meet the demand of energy. The proteins were the alternatives as in the present study which substantiate the toxic action.

Majumdar and Anilava (2019) [27], chlorpyrifos, as a toxicant to the fish *Oreochromis niloticus* reported 90 µg/L for technical grade and 20% EC 42 µg/L as 96 h value and the study was static only. The fish liver tissue had glycogen depletion, alkaline phosphatase, AChE, and catalyses too, decreased whereas in liver of plasma glucose, AAT and ALAT increased due to which the toxic action was caused as a result of toxic stress metabolism of glycolysis got enhanced and also gluconeogenesis paved the way of alternative energy source for energy source. This same might by the reason variations of them also, as in the present study for the death of the snake headed fish.

Anita Bhatnagar *et al*. (2016) [28] reported that in the fish *Cirrhinus mrigala* due to intoxication of the toxicant EC 50% of chlorpyrifos, by static test, 0.44 mg/L^{-1} as 96 h LC_{50} value. The disruption of the nucleus, as MN study responsible for the toxic action hence the resultant toxicity. Fish is varied of the present study, the value cannot be compared.

In the review article Deb and Das (2013) [9] mentioned that toxicity value varied and was evident that, species specific. The review article gave a list of species with their toxicity values, which can be summarised as 96 hrs LC_{50} value which is as follows:

S.No.	**Name of the fish**	**% TG**	**96 hr value**
1	*Lepomis macrochinus* (Blue gill sunfish)	98	3.3 ppb
2	*Onchorynchus mykiss* (Rainbow trout)	98	3.0 ppb
3	*Ictalarus punctata* (Channel catfish)	98	13.4 ppb
4	*Salvenius namaycush*	97	(a)140 ppb (pH 6.0) (b)98 ppb (pH 7.5) (c)205 ppb (pH 9.0)
5	*Salmo clarki* (Cutt trout trout)	97	(a)18.4 ppb (pH 7.5) (b)5.4 ppb (pH 9.0)
6	*Pimephales promelas* (Fat head minnow)	99.9	203 ppb
7	*Notemigonus crysolencas* (Golden shiner)	99	35 ppb
8	*Oreochromis niloticus*	Juvenile Adult	98.67 µg/L 154.01 µg/L
9	*Poccilia reticulate*		0.176 ppm
10	*Gambusia affinis*		2.97 mg/L
11	*Cyprinus carp* (Common carp)		5.80 mg/L

From the above information, the toxicity values very with fish, pH and technical grade percent composition of the chlorpyrifos. In the present study, the pH of the water used is 8.1 and percent of technical grade is 98% and the method of study includes both static renewal as well as CFTS. Hence, the values can be considered separately for the size of the fish, in the specific pH of the water and test method employed.

Mahdi Banee *et al*. (2013) [29] reported for 40.8% EC of the chlorpyrifos (Available only in that region) for the fish *Cyprinus carpio* and the method was static renewal, 860.87 µg/L, 452.18µg/L, 277.65 µg/L and 202.78µg/L for 24,48, 72 and 96 h respectively. Biochemical alterations such as AAT, ALAT, LDH, AChE, ALP and CPK all resulted in the blood due to the toxic action that exerted.

Ramesh and Saravanan (2008) [30] reported that the fish *Cyprinus carpio*, with a median lethal concentration as 5.28 ppm by static method, for a period of 24 hrs. They concluded that the haematological changes that took place in the fish blood caused the toxic action which even in the present study might be the possible reason. Ramesh and David (2009) [31] too reported the 96 hr value of *Cyprinus carpio* as 0.160 mg/L where the fish showed the signs of behavioural changes.

The National Pesticide Information Centre (NPIC) (2011) [32] reported that chlorpyrifos is toxic to fish, quoting Kamrin (1997) [33]. AChE inhibition like that of the injects was the causative factor of toxicity. Auta and Ogueji (2008) [34] reported on the toxicity of chlorpyrifos to the fish *Clarias gariepinus*. They reported that 96 hr value for the fish by static test was 0.92 mg/L and the mainly behavioural attributes are the reasons of toxicity as indices that showed physiological alterations and respiratory changes.

Tilak *et al.* (2004) [35] and Tilak and Koteswara Rao (2003) [36] reported the lethal concentrations, LC_{50} values for the three fish, *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* by both static and CFTS method. The values of 24 hrs in static test for *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* as 510 µg/L, 740 µg/L, 940 µg/L respectively and in CFTS 460µg/L, 580µg/L, 850µg/L, respectively. The values decreased as the time progressed, from 24 to 96 hrs and static values were reported to be higher than CFTS *Catla catla* is more sensitive of all the three carps, Cirrhinus is more resistant and Labeo was in between Respiratory, haematological, histopathological and biochemical changes, all due to the toxic stress only that caused the toxic action.

Van Wingaadhan *et al*. (1993) [37] reported the toxicity values of *Gastrostrees aculeatus* and also for *Pungitius pungitus* for 48 hrs and 96 hrs as 4.5 µg/L, 3.8 µg/L and 13.4 µg/L and 8.5 µg/L respectively for chlorpyrifos (4% Durban, EC by altogether or different methodology (single species toxicity tests) which not gained much prominence.

Fig.1. Graphical representation of 24h LC_{50} values in Static renewal system for Technical grade of Chlorpyrifos in the fish *Channa punctata*

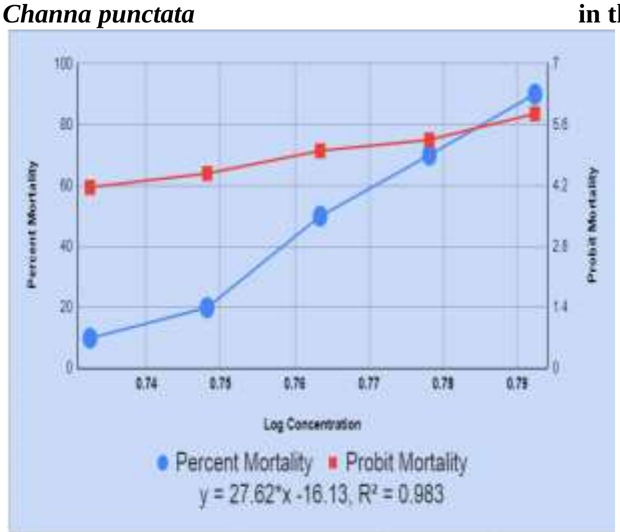


Fig.2. Graphical representation of 48h LC_{50} values in static renewal system for Technical Grade of Chlorpyrifos in the fish *Channa punctata*

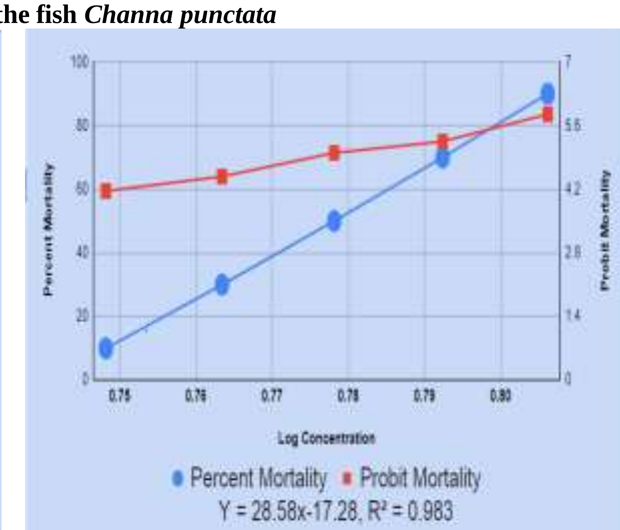


Fig. 3. Graphical representation of 72h LC_{50} values in Static renewal system for Technical grade of Chlorpyrifos in the fish *Channa punctata*

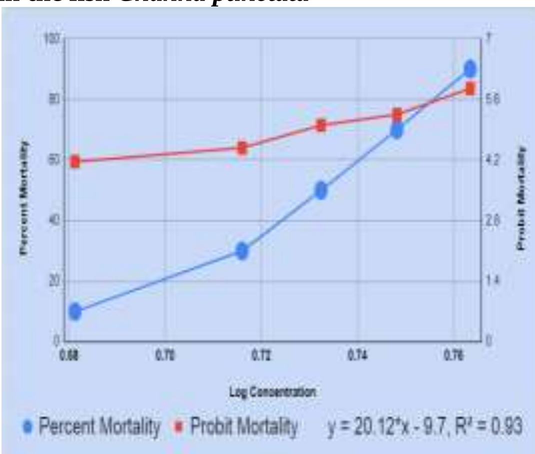


Fig. 4. Graphical representation of 96h LC_{50} values in Static renewal system for Technical grade of Chlorpyrifos in the fish *Channa punctata*

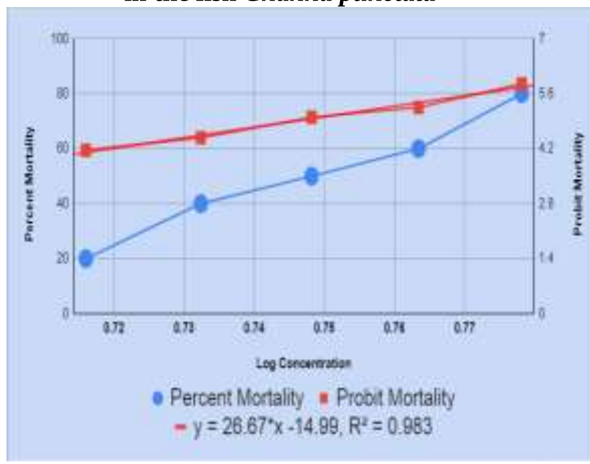
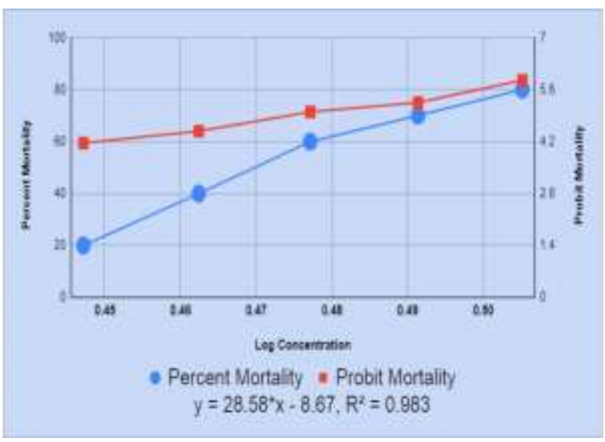


Fig. 5. Graphical representation of 24h LC_{50} values in Static renewal system for 20% E.C. of Chlorpyrifos in the fish *Channa punctata*

Fig.6. Graphical representation of 48h LC50 values in static renewal system for 20% E.C. of Chlorpyrifos in the fish *Channa punctata*



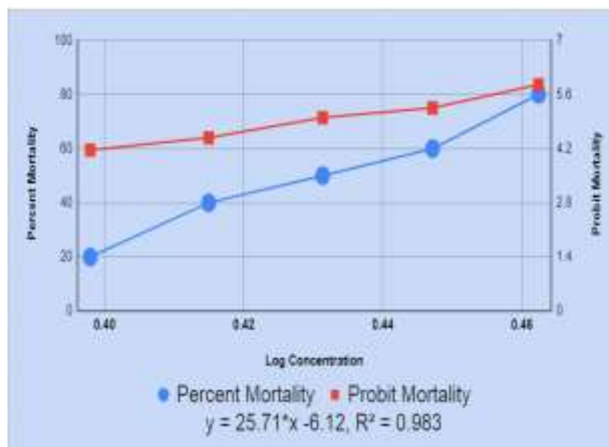


Fig. 7. Graphical representation of 72h
Fig.8. Graphical representation of 96h
LC_{50} values in Static renewal system for 20% E.C. of Chlorpyrifos in the fish *Channa punctata*

LC_{50} values in Static renewal system for 20% E.C. of Chlorpyrifos in the fish *Channa punctata*

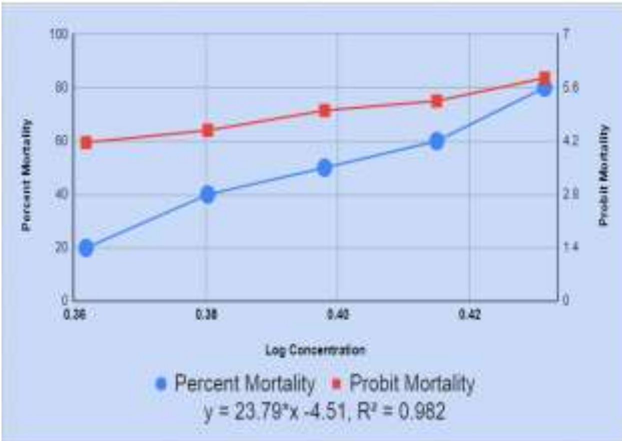


Fig. 9. Graphical representation of 24h LC_{50} values in Continuous Flow through system for Technical grade of Chlorpyrifos in the fish *Channa punctata*

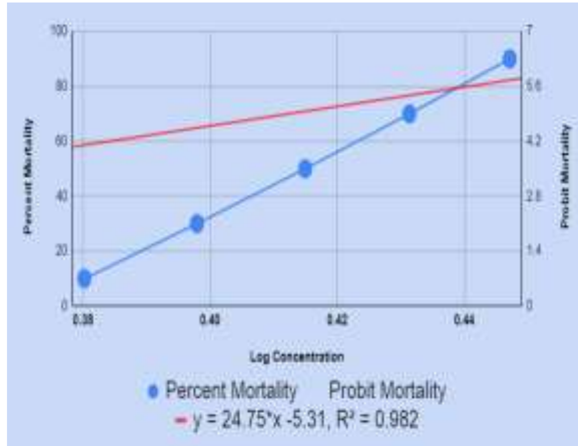


Fig.10. Graphical representation of 48h LC_{50} values in Continuous Flow through system for Technical Grade of Chlorpyrifos in the fish *Channa punctata*

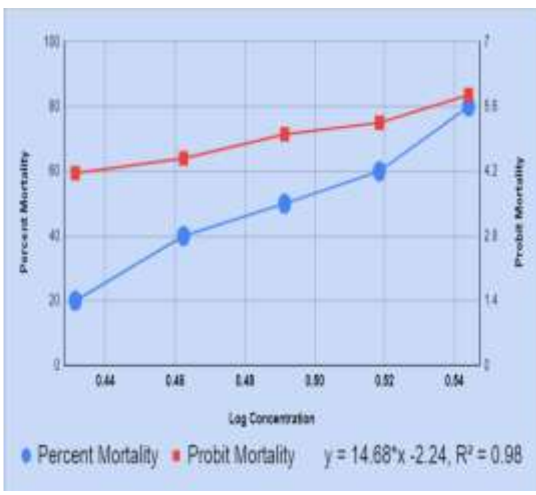


Fig. 11. Graphical representation of 72h LC_{50} values in Continuous Flow through system for Technical grade of Chlorpyrifos in the fish *Channa punctata*

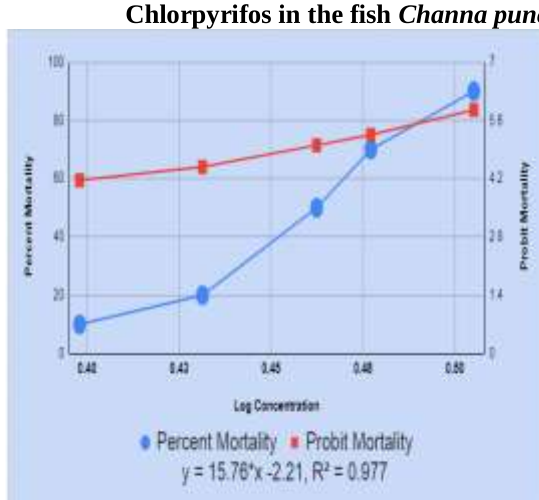
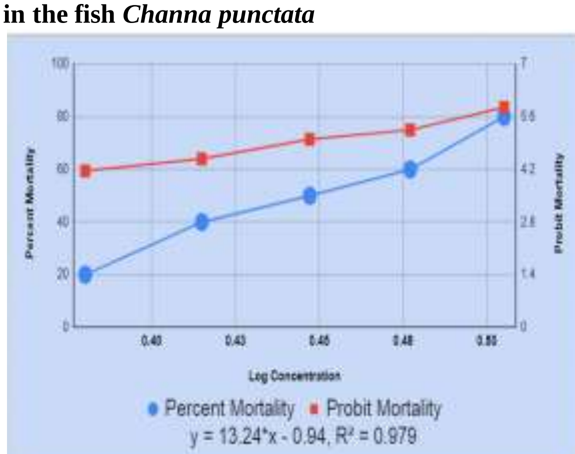


Fig. 12. Graphical representation of 96h LC_{50} values in Continuous Flow through system for Technical grade of Chlorpyrifos in the fish *Channa punctata*



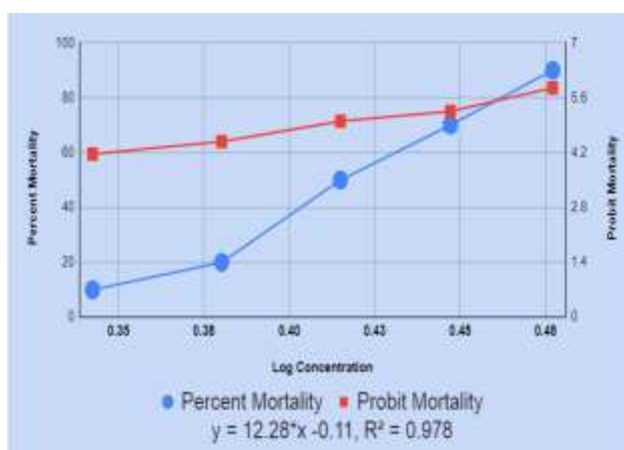


Fig.13. Graphical representation of 24h LC_{50} values in Continuous Flow through system of 20% EC, Chlorpyrifos in the fish *Channa punctata*

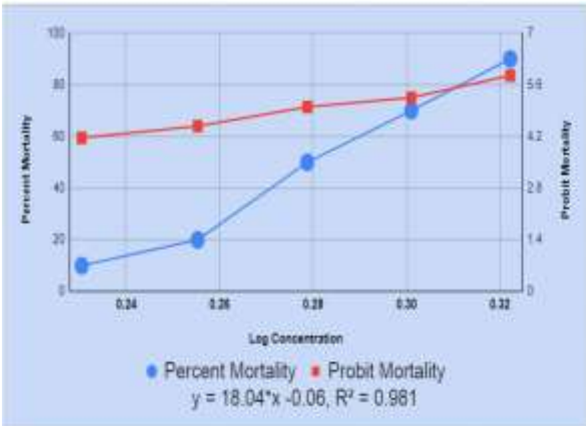


Fig.14. Graphical representation of 48h LC_{50} values in Continuous Flow through system of 20% EC, Chlorpyrifos in the fish *Channa punctata*

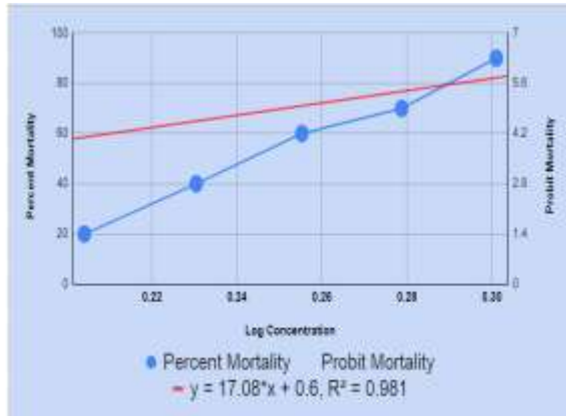


Fig.15. Graphical representation of 72h LC_{50} value in Continuous Flow through system for 20% EC, Chlorpyrifos in the fish *Channa punctata*

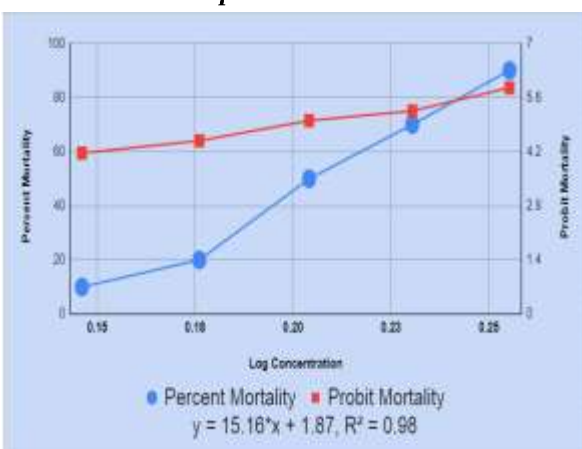


Fig.16 Graphical representation of 96h LC_{50} value in Continuous Flow through system for 20% EC, Chlorpyrifos in the fish *Channa punctata*

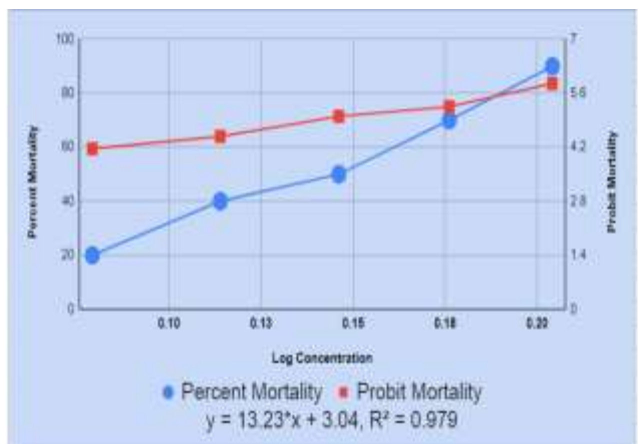


Table 1:- 24h LC_{50} values, Regression equation, slope intercept and R^2 value of chlorpyrifos technical grade and 20% EC in static renewal and continuous flow through system (CFTS) in the freshwater fish *Channa punctata*.

LC_{50} (µg/L)				**Regression Equation, slope, Intercept and R^2 value**			
Static renewal		**Continuous flow through system (CFTS)**		**Static renewal**		**Continuous flow through system (CFTS)**	
Technical grade	**20% EC**	**Technical grade**	**20% EC**	**Technical grade**	**20% EC**	**Technical grade**	**20% EC**
6.02 (5.62-6.44)*	3.01 (2.81-3.22)*	2.97 (2.15-4.12)*	1.91 (1.71-2.13)*	y=28.59x - 17.28 (28.59)[1]	y=28.59x - 8.68 (28.59)[1]	y=5.98x - 2.18 (5.98)[1]	y=18.05x + 0.06 (18.05)[1]

				(-17.28)[2] (0.98)[3]	(-8.68)[2] (0.98)[3]	(-2.18)[2] (0.75)[3]	(-0.06)[2] (0.98)[3]

* values in the parentheses are 95% fiducial confidence limits
1. Value in the parenthesis is slope
2. Value in the parenthesis is Intercept
3. Value in the parenthesis is R^2

Table 2:- 48h LC_{50} values, Regression equation, slope intercept and R^2 value of chlorpyrifos technical grade and 20% EC in static renewal and continuous flow through system (CFTS) in the freshwater fish *Channa punctata*.

LC_{50} (µg/L)				**Regression Equation, slope, Intercept and R^2 value**			
Static renewal		**Continuous flow through system (CFTS)**		**Static renewal**		**Continuous flow through system (CFTS)**	
Technical grade	**20% EC**	**Technical grade**	**20% EC**	**Technical grade**	**20% EC**	**Technical grade**	**20% EC**
5.82 (5.42-6.25)*	2.71 (2.51-2.92)*	2.87 (2.54-3.25)*	1.81 (1.61-2.03)*	y=27.63x - 16.13 (27.63)[1] (-16.13)[2] (0.98)[3]	y=25.71x - 6.13 (25.71)[1] (-6.13)[2] (0.98)[3]	y=15.76x - 2.22 (15.76)[1] (-2.22)[2] (0.98)[3]	y=17.09x + 0.61 (17.09)[1] (-0.61)[2] (-0.98)[3]

* values in the parentheses are 95% fiducial confidence limits
1. Value in the parenthesis is slope
2. Value in the parenthesis is Intercept
3. Value in the parenthesis is R^2

Table 3:- 72h LC_{50} values, Regression equation, slope intercept and R^2 value of chlorpyrifos technical grade and 20% EC in static renewal and continuous flow through system (CFTS) in the freshwater fish *Channa punctata*.

LC_{50} (µg/L)				**Regression Equation, slope, Intercept and R^2 value**			
Static renewal		**Continuous flow through system (CFTS)**		**Static renewal**		**Continuous flow through system (CFTS)**	
Technical grade	**20% EC**	**Technical grade**	**20% EC**	**Technical grade**	**20% EC**	**Technical grade**	**20% EC**
5.62 (5.23-6.05)*	2.61 (2.41-2.82)*	2.81 (2.43-3.26)*	1.61 (1.41-1.83)*	y=26.67x - 14.99 (26.67)[1] (-14.99)[2] (0.98)[3]	y=24.76x - 5.31 (24.76)[1] (-5.31)[2] (0.98)[3]	y=13.24x + 0.94 (13.24)[1] (-0.94)[2] (0.96)[3]	y=15.67x + 1.88 (15.67)[1] (-1.88)[2] (0.98)[3]

* values in the parentheses are 95% fiducial confidence limits
1. Value in the parenthesis is slope
2. Value in the parenthesis is Intercept
3. Value in the parenthesis is R^2

Table 4:- 96h LC_{50} values, Regression equation, slope intercept and R^2 value of chlorpyrifos technical grade and 20% EC in static renewal and continuous flow through system (CFTS) in the freshwater fish *Channa punctata*.

LC_{50} (µg/L)				**Regression Equation, slope, Intercept and R^2 value**			
Static renewal		**Continuous flow through system (CFTS)**		**Static renewal**		**Continuous flow through system (CFTS)**	
Technical grade	**20% EC**	**Technical grade**	**20% EC**	**Technical grade**	**20% EC**	**Technical grade**	**20% EC**
5.38 (4.89-5.93)*	2.51 (2.31-2.72)*	2.61 (2.23-3.06)*	1.41 (1.21-1.63)*	y=20.13x - 9.71 (20.13) [1] (-9.71) [2] (0.93) [3]	y=23.80x - 4.51 (23.80) [1] (-4.51) [2] (0.98) [3]	y=12.28x - 0.12 (12.28) [1] (-0.12) [2] (0.98) [3]	y=13.24x + 0.30 (13.24) [1] (-3.04) [2] (-0.98) [3]

* values in the parentheses are 95% fiducial confidence limits
1. Value in the parenthesis is slope
2. Value in the parenthesis is Intercept
3. Value in the parenthesis is R^2

Conclusions:-

Thus when the toxicant find its presence in the ambient waters the fresh water fish are subjected to toxic action. If the concentrations are above the permissible levels and such lethal concentration make the fish to have acute toxicity. Otherwise, if it is in sublethal concentration, the fish had other effects which is not desirable. The commercial formulation must have strict vigilance via the quality control because the ingredients which are causing additional/cumulative toxicity, the user have no other option to use to combat pests which are cheaper, low persistence but must of the good quality.

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