

Assessment of Erymax30 in-vivo LC50 Reference Values in Snake-headed murrel (*Channa punctatus* Bloch)

Om Prakash Kumar¹, Mohita Sardana^{2*}, Sangeeta Kumari³ and Amit Priyadarshi⁴

^{1,2*,3,4}Department of Zoology, Veer Kunwar Singh University, Arrah – 802 301

*Corresponding author: Mohita Sardana

*Email: mohita.sardana23@gmail.com

KEYWORDS

Channa punctatus, LC50, Erymax 30, Acute toxicity.

ABSTRACT

The *in-vivo* acute toxicity, median lethal concentration, and safe concentration of Erymax 30 in an Indian snake-headed murrel, *Channa punctatus* (Bloch), weighing 50-60g, were assessed using various methodologies. Lorke and Enevide et al. estimated the 96-hour LC50 value as 189.7 and 190.0 mg/l, respectively. As an approximate estimate, the Up-and-Down approach produced a 96-hour LC50 range of 200.0 to 210.0 mg/L. Furthermore, the Behren-Karber, Miller, Tainter, and Finney techniques reported 96-hour LC50 values of 222.0, 205.17, and 192.75mg/l, respectively. Finally, the application of the Reed-Muench method calculated the 96-hour LC50 dose and the median optimum dose of 208.45mg/l. In *Channa punctatus*, the cumulative median lethal average of Erymax 30 at 96 hours was $222.0+205.17+192.75+208.45=207.09$ mg/l. The doses of Erymax 30 have a significant relation to fish mortality. According to the data, Erymax 30 accumulates in aquatic bodies to disrupt this fish's physiological activity. As a result, while treating *Channa punctatus* with high dosages of Erymax 30, proceed with caution.

Channa punctatus is less susceptible to Erymax 30 toxicity than other fish. The computed safety level value revealed that identifying an acceptable dose of Erymax 30 was possible. Erymax 30 could be considered a moderately/highly hazardous drug to *Channa punctatus*.

1. INTRODUCTION

In recent years, poor management of fish farms and intensely cultivated fisheries has resulted in infectious diseases among fish. Disinfection, chemotherapy, and antibiotic use are among the many methods available for disease control in fish farms ⁽¹⁾.

Antibiotics are widely used for sanitation and health maintenance ⁽²⁾. According to Ben et al. ⁽³⁾, 80% of antibiotics end up in the aquatic environment in their natural form. Furthermore, investigations have revealed that antibiotics are commonly found in underwater systems ⁽⁴⁾. Antibiotics are typically identified in rivers and wastewater at levels of ug/L⁽³⁾. Previous research has identified antibiotic concentrations at mg/l levels in swine effluent ⁽⁵⁾, Erythromycin is frequently used in livestock production ⁽⁶⁾. Antibiotics are regarded as a substance with long-term negative effects on the natural environment ⁽⁷⁾. Antibiotics have been shown to delay the hatching of fish eggs ⁽⁸⁾, damage gills and liver ⁽⁹⁾, and destroy antioxidant defences in muscle, which affects fish metabolism and injures neurons ⁽¹⁰⁾. Erythromycin, a semisynthetic antibiotic with bacteriostatic properties, has been widely utilized since the 1950s. Because sewage treatment facilities do not effectively dispose of organic contaminants, erythromycin is distributed in the aquatic environment. It takes a long time for erythromycin to break naturally ⁽¹¹⁾; in addition, prior research has revealed that erythromycin has detrimental effects on fish⁽¹²⁾. Kulveret al.⁽¹³⁾ focused on the physiological effects on fish, rather than the harmful effects on behavior. Acute toxicity is defined as an unfavorable outcome that occurs within 24 hours of single or repeated toxicant treatments ⁽¹⁴⁾. The median lethal concentration (LC50) has been utilized as an important criterion in measuring acute toxicity and as an initial screening process to identify the toxicity of a chemical ^(15,16). *Channa punctatus* (Bloch), a freshwater Indian Snake-headed murrel, is one of the most common fish. In many Indian university laboratories, it is employed as a model for dealing with a teleost's alimentary canal and a test animal. Erymax 30 has not been examined in Indian air-breathing fishes ^(17, 18). The study sought to ascertain the median lethal dose and safe level of Erymax 30 on *Channa punctatus*. The research will determine the toxicity level; and will be useful in determining differences in fish health caused by Erymax 30 toxicity, the suitability of environmental conditions for fish, the relative sensitivity of fish, and the physicochemical conditions of water bodies.

2. MATERIALS AND METHODS

The investigation was carried out in a laboratory setting, using the ethics of the Department and VKS University, Arrah, using a static renewable method.

During March-April 2023, live healthy specimens of *Channa punctatus* (Bloch) (BW: 50.0-60.0g) were purchased from Arrah (Bhojpur), Bihar's local market. Before being transferred to large aquaria, the fish were disinfected with dilute KMnO₄. The goat liver was fed fish, and other food items were purchased from the local market. Following APHA⁽¹⁹⁾, they acclimatized for a fortnight at VKS University Departmental Laboratory in Arrah.

In this experiment, we used Erymax 30 for fish (GOOVET). The dose schedule was based on preliminary research with a variety of doses and a previous report of Erymax 30 in fish (Kasagala and Pathiratne, 2008). Twenty specimens were fed Erymax 30 (1, 10, 100, 1000 mg/l) and another set of controls (Table 2). Lockwood⁽²⁰⁾ defined death as total immobility with no abdominal flexion upon forced extensions.

Erymax 30 toxicity was classified using a standard table⁽²¹⁾. We follow established standards to determine the safe dosage of Erymax 30⁽²²⁻²⁷⁾.

The data was entered into a Microsoft Excel spreadsheet. The observations were averaged for each group in the analysis using group analysis and linear regression. For statistical evaluation, Graph Pad Prism 5 software was used.

3. RESULTS AND DISCUSSION

Physicochemical characteristics of water: The physicochemical characteristics of test water having a temperature of 25.0±1.0°C, pH: 7.22±0.18, dissolved oxygen: 6.4±0.08 mg/l, total alkalinity: 73.0±4.2 mg/l, hardness: 230.67±55.26 mg/l and chloride: 16.7±0.2 mg/l was recorded during the experimental period (Table 1). The observed parameters are within the range of favorable growth performances⁽²⁸⁾. Physiological parameters such as antibiotic concentration, formulation, and exposure impact studies⁽²⁹⁾.

Ethological observations: Different doses of Erymax 30 resulted in uncoordinated behavior in the fish. In response to sudden changes in the surrounding environment, fish were alert, stopped swimming, and remained static in position during preliminary exposure. After a while, they tried to avoid the toxic water by swimming and jumping quickly. Because of the stretching of their body muscles, their fins became tough and stretched. They secreted massive amounts of mucus from their entire body, which deposited inside the buccal cavity and gills. The pigmentation of the body increased. Finally, the fish exhibited vertical posture for a few minutes, with its mouth close to the water's surface to gulp air and its tail pointing downward. They soon settled at the bottom of the tank, and after a while, their bellies grew upward, and the fish died while the opercula remained wide open, exposing the gills.

Stress-induced behavioral changes are the most sensitive indicator of potential toxic effects. The numerous behavioral changes observed in *Channa punctatus*, such as restlessness, abnormal swimming behavior, vigorous jerks, loss of balance, myotonia, and anorexia, are similar to previous observations in fish exposed to various toxicants^(30, 31).

Determination of Dose

(1) Lorke⁽³²⁾ and Enegetet al.⁽³³⁾ median lethal dose method. The method includes two stages.

(A) Three groups, each with three fish, formed from the nine fish. Each group of fish received a different dose of Erymax 30 (60, 120, and 180 mg/l). The fish are kept under observation for 96 hours to see how they behave and if they will die.

(B) Three fish are used in this stage, which is divided into three groups of one fish each. The fish are given higher doses of Erymax 30 (200, 300, and 400 mg/l) and then observed for 96 hours for behaviour and mortality.

Therefore, 96hr-LC50 = $\sqrt{C_0 \times C_{100}} = \sqrt{180 \times 200} = 189.74$ mg/l Erymax 30 (Table 3). Where, C₀ = the maximum concentration with no mortality of fish and C₁₀₀ = the minimum concentration with total mortality of fish.

Furthermore, 96hr-LC50 dose = $\frac{M_0 + M_1}{2} = \frac{180 + 200}{2} = 190.0$ mg/l (Table 3). Where, M₀ = maximum concentration with no mortality of fish and M₁ = minimum concentration with total mortality of fish.

(2) Dixon-Mood⁽³⁴⁾ Up-and-Down (Staircase) Method: The Erymax 30 is dosed sequentially to single fish in this method. Following the administration of the first dose, the next dose is determined by the outcome of the subsequent dose. If the fish survives the next dose, the dose is adjusted upward; if mortality occurs at the next dose, the dose is adjusted downward—a constant factor applied to change the dose either upward or downward. Testing is stopped when the upper limit (2000-5000 mg/l) is reached without mortality or when the LC50 is determined from the test. If a fish survives, a constant factor is added to the dose for the next fish and

vice versa. It estimates the MLC50 in 6 fish over 96 hours using arithmetic, geometric, and harmonic means. Using this method, MLC50 was calculated to be 205.0mg/l for Erymax 30 (Table 4) ^(35, 36).

(3) Behren⁽³⁷⁾-Karber⁽³⁸⁾ method: It is a non-parametric method requiring at least partial mortality, but the data does not fit the probit model. This method used equal spacing between dose intervals and an equal number of fish at each dose level for observations ranging from 0% to 100%. Many fish died because the calculated dose did not kill a single fish. During the first four hours, 24 hours, and daily for 04 days, fish were treated with the test substance and monitored for signs of lethality. The 96hr-LC50 dose of Erymax 30 is 222.0 mg/l in this method (Table 5).

(4) Regression analysis method: It is used when there is no partial mortality. The Erymax 30 doses were converted into a mortality rate. This method resulted in the death of many fish. Erymax 30 24, 48, 72, and 96hr-LC50 doses calculated using this method were 562.11, 299.23, 238.87, and 205.17 mg/l, respectively (Table 6). Erymax 30 dosage was gradually reduced during treatment.

(5) Finney's⁽³⁹⁾ probit analysis method: It is a parametric maximum likelihood method that calculates net/corrected percent mortality from 10% to 100%. Based on the straight line obtained in the graph, empirical probit ranging from 3.72 to 8.72 (Table-7) noted from the Fischer and Yates table. The expected/provisional probit was assessed using empirical probit values ranging from 3.72 to 8.72, and the working probit (from 3.82 to 6.64) and weighing coefficient (from 0.238 to 0.614) values were calculated to determine the mean and deviation of the Erymax 30 doses and mortality. Finally, Erymax 30 median lethal concentration was 96hr-LC50 = Antilog 2.285 = 192.75 mg/l. Because the determined value of χ^2 is less than the tabulated value, the data is homogeneous, indicating that the 'eye fit' line is in good agreement.

(6) Reed-Muench⁽⁴⁰⁾ method: If the dose is not spaced symmetrically around log LC50, it causes a bias in LC50 estimation. This study requires experimentation with 10 fish per dose for a better correlation. Furthermore, the smallest test dose must kill one fish, and only four test doses are permissible. Finally, the method tweaks the calculation of the percentage of test animals that lived and died⁽⁴¹⁾. The 95% confidence level could not be determined using the applied method (Table 8).

According to Reed-Muench's⁽⁴⁰⁾ method, the median lethal dose of Erymax 30 is 208.45 mg/l. It demonstrated the arithmetic mean precision, validity, and dependability as a rough estimate of LD50. The geometric mean is used in toxicological studies for exponential data where an arithmetic mean is incorrect, such as rates and ratios; harmonic mean is used ^(42, 43). As a result, for a rough estimate of LD50 dose, arithmetic or harmonic mean can be used instead of geometric mean.

The 96hr-LC50 calculated using the Behren⁽³⁷⁾-Karber⁽³⁸⁾ method, the regression analysis method, and the Finney probit analysis method are nearly identical. These are the most common methods for determining 96hr-LC50 used by workers. The Behren-Karber method, on the other hand, can be used to calculate the 24, 48, 72, and 96hr-LC50 doses from the same set of experiments.

(7) Chinedu et al.⁽⁴⁴⁾ method: it is divided into three stages. The results of each stage determine the next step. The fish should be observed for 1 hour after administration, followed by 10 minutes every 2 hours for the next 24 hours. Toxic behavioral signs and mortality should be recorded (Table 9).

Stage 1 or Initial Stage: Four fish are required. The fish were divided into four groups, each containing one fish. The fish were subsequently administered doses of Erymax 30. No mortality is recorded at this stage, so we move to stage 2.

Stage 2: Three fish are involved in this stage, which is divided into three groups of one fish each. Different Erymax 30 doses were higher than those used in stage 1. Because there was no death, the test proceeded to stage 3.

Stage 3: Three fish are needed for this stage, divided into three groups, with one fish in each group. The different fish groups received Erymax 30 doses ranging from 3000 to 5000 mg/l. Mortality rates were documented for each dosage. When no mortality is recorded at this stage, the Erymax 30 LC50 is said to be greater than 5000 mg/kg, indicating a high level of safety.

Confirmatory (or confidence) test

If mortality was recorded at a given dose in any of the stages, a confirmatory test should be performed to ensure that the Erymax 30 was the cause of the death.

This test involves administering Erymax 30 at the dose that caused death in previous trials or the lowest dose that resulted in death if multiple doses caused fatalities. During the next 24 hours, observe the animals for one hour after administration, and then for 10 minutes every two hours. If either of the two animals dies during this period, confirm the test result.

This account suggests that differences in LC50 values reflect differences in the sensitivity of different fish species to various types of toxicant exposures. It appears to be influenced by the type and age of fish used in the toxicity tests. However, according to Pandit and Kumari (2020), the average 96hr-LC50 Terramycin 500 dose for *Clarias batrachus* 663.8 mg/l. Therefore, the current study supports the earlier findings^(17, 45).

There was a significant relationship between the dose of Erymax 30 and fish mortality. According to these findings, *Channa punctatus* is more resistant to Erymax 30 toxicity than most other fishes. The findings are consistent with previous research. The LC50 dose difference between species follows the body weight, age, sex, and feeding conditions of the test fishes, ambient conditions, water temperature, and regional variations. The shape, size, surface area, surface charges, and chemical composition influence the toxicity of Erymax 30. The acute toxicity study has indicated that the rate of deaths for a fixed time increases with an increase in concentration and for a particular concentration with an increase in exposure time⁽⁴⁶⁾. The death of fish in higher concentrations of Erymax 30 may be due to hypoxemia or impaired oxygen uptake by the gills. While in lower concentrations, the slow intrusion of the drug might have primarily induced alterations in physiology and ultimately led to death. The vital cause of mortality might be the damage of the respiratory epithelium by oxygen consumption during the formation of mucus covering the gills of fish⁽⁴⁷⁾.

A comparison of the Lorke⁽³²⁾, Up and Down, Karber⁽³⁸⁾, and Chinedu et al.⁽⁴⁴⁾ methods revealed that the Chinedu et al.⁽⁴⁴⁾ method is superior in duration, expenditure, number of fish required, and nature of results (Table 10). The cumulative median lethal concentration (96 hr-LC50) for Erymax 30 in *Channa punctatus* was calculated using the Behren⁽³⁷⁾ and Karber⁽³⁸⁾ methods, regression analysis, Finney's⁽³⁹⁾ probit analysis method, and the Reed-Muench⁽⁴⁰⁾ method. The overall result is $222.0 + 205.17 + 192.75 + 208.45 = 207.09$ mg/l. Erymax 30 is considered to be moderately to highly toxic to *Channa punctatus*, based on the available data.

Safe levels are reported to account for uncertainties in data and evaluation processes. In the absence of acute toxicity data, the safe level is also employed. Erymax 30 safety level is 11.10 (2.99 to 21.72) mg/l (Tables 11 and 12) for *Channa punctatus*. Furthermore, Erymax 30 has a safe level of 1.110 mg/l in rats and 0.1110 mg/l in humans (<https://en.wikipedia.org/wiki/Toxicity>).

4. CONCLUSION

Determining the LC50 dose is a crucial step in ecotoxicological research. The method proposed by Lorke and Enegide et al., followed by the Up-and-Down method, is applicable for range finding and provides a rough estimate of the LC50 dose for improved results. The LC50 dose should then be calculated using the Behrens-Karber method or regression analysis with the Finney probit method. Cross-checks suggested by Chinedu et al. should be employed to confirm the value of the LC50 dose.

The current study indicates that establishing the acceptable concentration of Erymax 30 in *Channa punctatus* is challenging due to the range of safe levels. However, Erymax 30 is moderately/highly toxic to *Channa punctatus* depending on the toxicity range.

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Table-1. Certain physico-chemical features of experimental water.

S. No.	Physico-Chemical features	Value
1.	Temperature	25.0±1.0°C
2.	pH	7.22±0.18
3.	Dissolved oxygen	6.04±0.08 mg/L
4.	Free Carbon dioxide	1.43±0.58 mg/L
5.	Hardness	230.67±55.26mg/L
6.	Total alkalinity	73.0±4.2mg/L
7.	Chloride	16.7±0.2mg/L

Table-2. Range finding of toxicity of Erymax to *Channa punctatus* (body weight: 50-60g).

S. No.	Concentration of sodium fluoride (mg/L)	Percentage of mortality
1.	1.0	0
2.	10.0	0
3.	100.0	3
4.	1000.0	100

Table 3. Lorke's (1983) method for LC₅₀ dose of Erymax 30 for *Channa punctatus* (body weight: 55.0g)

S. No.	Dose of Erymax (mg/L)	Log dose of Erymax (mg/L)	96hr % mortality (n = 3 in each group)	S. No.	Dose of Erymax (mg/L)	Log dose of Erymax (mg/L)	96hr % mortality (n = 1 in each group)
1.	60	1.778	0	1.	400	2.602	1
2.	120	2.079	0	2.	300	2.477	1
3.	180	2.255	0	3.	200	2.301	1

Lorke's (1983) method: $96hr-LC_{50} = \sqrt{C_0 \times C_{100}} = \sqrt{180 \times 200} = 189.74mg/L$ Erymax 30.

Engide et al, (2013) method: $96hr-LC_{50} = \frac{M_0 + M_1}{2} = \frac{180+200}{2} = 190.0mg/L$ Erymax 30.

Table 4: Up-and-Down method (Bruce, 1985) for determination of LC₅₀ dose of Erymax 30 for *Channa punctatus* (body weight: 55.0g)

Number	Dose (mg/L)	Survival/Mortality	Number	Dose (mg/L)	Survival/Mortality
1 st	120	Survival	1 st	300	Mortality
2 nd	180	..	2 nd	260	..
3 rd	240	Mortality	3 rd	220	..
4 th	180	Survival	4 th	180	Survival
5 th	240	Mortality	5 th	140	..
6 th	300	..	6 th	100	..
Arithmetic Mean	210.0	LC ₅₀	Arithmetic Mean	200	LC ₅₀
Standard deviation	62.9381	1.79 (Default dose)	Standard deviation	74083	1.97 (Default dose)
Standard error of Mean	14.78	210.0± 14.78 (Confidence interval)	Standard error of Mean	17.58	200±17.58 (Confidence interval)
Geometric Mean	201.63	LC ₅₀	Geometric Mean	187.35	LC ₅₀
Harmonic Mean	192.86	LC ₅₀	Harmonic Mean	174.30	LC ₅₀

$$\text{Mean LC}_{50} = \frac{210.0+200.0}{2} = 205.0\text{mg/L Erymax 30.}$$

Table – 5: Behren-Karber method for 96hr-LC₅₀ determination of Erymax30 for *Channa punctatus* (body weight: 55.0g)

Group	Dose of Erymax (mg/l)	Difference between two consecutive dose (A)	No. of fish exposed	Mortality				Overall mortality at 96hr	Mean mortality between two consecutive dose (B)	A x B
				24hr	48hr	72hr	96hr			
1	0	0	10	0	0	0	0	0	0	0
2	160	80	10	0	0	0	1	1	0.5	40
3	200	40	10	0	1	2	4	4	2.5	80
4	240	40	10	1	3	6	7	7	5.5	180
5	280	40	10	3	6	8	9	9	8.0	300
6	320	40	10	6	7	9	10	10	9.5	380
										980.0

$$\begin{aligned} 96\text{hrLC}_{50} &= \text{LC}_{100} - \frac{\Sigma AB}{N} \\ &= 320 - \frac{980}{10} \\ &= 320 - 98 \\ &= 222 \text{ mg/L of Erymax 30} \end{aligned}$$

Table – 6: Statistical relationship between dose of Erymax 30 (mg/L) and mortality of *Channa punctatus* (body weight: 55.0g)

Sl. No.	Exposure period (hours)	Regression equation $y = bx + a$ (% mortality)	Lethal Concentration (mg/L)		Toxicity Factor	t value (df=5)	F value (u = 1; v = 4)	95% Confidence limit	
			LC ₁₀	LC ₅₀				Lower	Higher
1	24	$y = 0.106x - 9.583$	LC ₁₀	184.74	1.000	1.964 (p<0.01)	3.857 (p<0.05)	44.54	414.01
			LC ₅₀	562.11				135.51	1259.73
			LC ₉₀	939.49				226.86	2105.46
2	48	$y = 0.22x - 15.83$	LC ₁₀	117.41	1.878	2.637 (p<0.01)	6.954 (p<0.01)	28.30	261.03
			LC ₅₀	299.23				72.14	670.60
			LC ₉₀	481.05				115.97	1078.07
3	72	$y = 0.30x - 21.66$	LC ₁₀	105.53	2.356	2.613 (p<0.01)	6.828 (p<0.01)	25.44	236.50
			LC ₅₀	238.87				57.58	535.23
			LC ₉₀	372.20				89.73	834.13
			LC ₁₀	84.32		3.567	12.72	20.33	188.97

4	96	$y = 0.331x - 17.91$	LC ₅₀	205.17	2.730	(p<0.001)	(p<0.01)	49.46	459.80
			LC ₉₀	326.01				78.59	730.62

Table 7: Probit analysis for toxicity of Erymax 30 in *Channa punctatus*

Dose of Erymax(mg/L)	Log dose of Erymax (mg/L)	Number of fish exposed	Mortality of fish	% of mortality of fish	Net/corrected mortality of fish	Empirical probit	Expected/Provisional probit	Working probit	Weighing Coefficient	nw	nwx	nwy	nwx ²	nwy ²	nwxy
x	n		p			Y	y	w							
0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
160	2.204	10	1	10	10	3.72	3.42	3.82	0.238	2.38	5.245	9.10	11.56	34.73	17.94
200	2.301	10	4	40	40	4.75	4.75	4.72	0.614	6.14	14.128	28.98	32.51	136.79	66.68
240	2.380	10	7	70	70	5.52	5.54	5.48	0.564	5.64	13.423	30.91	31.95	169.37	73.56
280	2.447	10	9	90	90	6.28	6.32	6.34	0.334	3.34	8.173	21.18	20.00	134.25	51.82
320	2.505	10	10	100	100	8.72	6.68	6.64	0.204	2.04	5.110	13.55	12.80	89.94	33.93
-	-	-	-	-	-	-	-	-	-	19.54	46.079	103.72	108.82	565.08	243.93

96hr-LC₅₀ = Antilog 2.285 = 192.75.6mg/L of Erymax 30.

Table – 8: Reed-Muench (1938) method for 96hr-LC₅₀ determination of Erymax 30 in *Channa punctatus* (body weight: 55.0g)

Sl. No.	Dose (mg/L)	Log dose (mg/L)	Experiment		Specific Cumulative			Rate of mortality	% mortality	% survival
			No of mortality	No. of survival	Mortality	Survival	Total			
1	160	2.204	1	9	3	36	39	$\frac{3}{39}$	7.70	92.30
2	200	2.301	4	6	7	10	17	$\frac{7}{17}$	41.18	58.82
3	240	2.380	7	3	14	4	18	$\frac{14}{18}$	77.77	22.23
4	280	2.447	9	1	21	1	22	$\frac{21}{22}$	95.45	4.55

Calculation of median lethal concentration (MLC ₅₀)	Calculation of median survival concentration (MSC ₅₀)
$\frac{50.0-41.18}{77.77-41.18} = \frac{8.82}{36.59} = 0.233$	$\frac{50.0-22.23}{58.82-22.23} = \frac{27.77}{36.59} = 0.759$
2.301 - 2.380 = 0.079	2.301 - 2.380 = 0.079
0.233 x 0.079 = 0.0184	0.759 x 0.079 = 0.060
2.301 + 0.0184 = 2.319	2.301 + 0.060 = 2.361
Antilog of 2.319 = 208.45 mg/L	Antilog of 2.361 = 229.61 mg/L
MLC₅₀ = 208.45mg/L	MSC₅₀ = 229.61 mg/L

Table 9: Recommended doses for the proposed method (Chinedu *et. al.* 2013)

Stages	Recommended doses (mg/L)			
	Group I	Group II	Group III	Group IV
1	10	100	300	600
	50	200	400	800
2	1000	1500	2000	-
3	3000	4000	5000	-

Table 10: Comparison of different methods

Sl. No.	Parameters	Lorke's method	Up and Down method	Karber's method	Chinedu method
1.	Duration	Less	May take more time	Less	Less
2.	Expenditure	Moderate	Less	High	Less
3.	No. of fishes	Few	Few	Much	Few
4.	Nature of experiment	Simple	Simple	Complicated	Simple
5.	Accuracy of results	Uncertain	Accurate	Uncertain	Accurate

Table 11: Classification of substances/toxicants on the basis of toxicity range (21, 48)

Sl. No.	Toxicity range (mg/L)	Toxicant Classification (21)	Sl. No.	Toxicity range (mg/L)	Toxicant Classification Environmental Protection Agency (48)
1.	<5	Extremely Toxic	1.	<50	Most Toxic
2.	5-50	Highly Toxic	2.	>50 and <500	Highly Toxic
3.	50-500	Moderately Toxic	3.	<5000	Moderately Toxic
4.	500-5000	Slightly Toxic	4.	>5000	Least Toxic
5.	5000-15000	Practically Non-toxic			
6.	>15000	Relatively Harmless			

Table 12: Estimation of safe levels of Erymax at 48/96hr exposure of *Channa punctatus* (body weight: 55.0g)

Sl. No.	Method	Dose of	Accumulation Factor	Safe level
		Erymax30 (mg/L)	Erymax 30 (mg/L)	Erymax30 (mg/L)
1.	Canadian Council of Resource and Environment Minister (=CCREM) (1991) and International Joint Commission (=IJJ) (1977)	96hr-LC50 = 205.17	0.05	205.17 x 0.05 = 10.26
2.	Committee on Water Quality Criteria (=CWQC) (1972)	48hr-LC50 = 299.23	0.01	299.23 x 0.01 = 2.99
3.	Hart et al, (1948)	96hr-LC50 = 205.17	0.03 $\times \left(\frac{24\text{hr-LC50}}{48\text{hr-LC50}}\right)^2$	205.17 x 0.03 x $\left(\frac{562.11}{299.23}\right)^2 = 21.72$
4.	National Academy of Sciences/ National Academy of Engineering (=NAS/NAE) (1973)	96hr-LC50 = 205.17	0.00001	205.17 x 0.00001 = 2.0517×10^{-3}
5.	Sprague (1971)	96hr-LC50 = 205.17	0.1	205.17 x 0.1 = 20.52
			Average	11.10