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# Evaluation Of Median Tolerance Limit ( Lc50 ) For A Fresh Water Fish Labeo Rohita Under The Stress Of Azodyes Metanil Yellow And Bismark Brown

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## ABSTRACT

*The present study is to evaluate the LC50 value of a fresh water fish Labeo rohita when exposed to azodyes Metanil yellow (4" Aniline azobenzene – m –sulfonic acid) and Bismark brown ( 2,4 – diamino 3" aminoazobenzene ). In case of metanil yellow the LC50 value at 96 hrs. Exposure was calculated to be 2.0414 Log mg/l and for bismark brown was calculated to be 2.000 Log mg/l at 96 hrs exposure.*

## INTRODUCTION

Qualitative aspects of toxicology are important because they are fundamental safety evaluation process in which, one first determine the toxicologic profile of the substance and this establishes how the chemical can be employed safely

to prevent injury (Plaa 1982). According to Durhan (1974 ) toxicity is the ability of a chemical molecule or a compound to produce injury once it reaches susceptible site which is determined by the dosage. Cairns (1984) reported that from the regulatory point of view, toxicity tests are usedfor three major purposes. They are 1- screening of chemicals and products 2- establishing limits and 3- monitoring. Bioassay tests can be used to establish the maximum acceptable concentration of a pollutant in a given environment without deliberate application of the chemical causing any unfavourable biological consequences.

The aim of using bioassay in monitoring of environmental pollution is to establish a relationship between toxicity and concentration of a pollutant being studied in a biotope.The toxic effects can be divided into two catagories viz. effects that occur very quickly after a brief exposure to a chemical agent (acute ) andthose that appear only after repetitive exposure to the substance (chronic ) (Durham 1974, Ramade 1987, Nagel 1993 ).

Toxicity can be calculated by determining the mortality rate after a fixed time as a function of increasing doses of the toxicants ( Ramade 1987 ) . The most important constant is the LC50( median lethal concentration ) which is the theoretical value causing 50% mortality in the population being studied.

### Material And Method Metanil Yellow

Metanil yellow (4'Aniline azobenzene – m- sulfonic acid), monoazo :C.I.Acid yellow 36, (13065) has been used as a colour additive to approximately 29% of food stuffs and ranks first among the nonpermitted colours. It is mainly used in colouring a wide variety of foodstuffs such

as rice, pulses, beverages, soft drinks, medicines etc. It is also used in dyeing cotton, wool, paper, soaps, waxes, polishes and some cosmetics. As a result of its large scale use, it is drained out to the inland water with the industrial discharge, in such an a the aquatic organism. This is the reason that it is selected as one of the toxicants in the present investigation.

**BISMARK BROWN**

The other toxicant selected for the present study is,Bismark brown (2.4 diamino 3' aminoazobenzene) (DAAB) a permitted basic aminoazodye. This dye is abundantly used for dying wool and silk fibres.

The coloured portion of the dye (present in the cotton) is derived from subsituted amino group such as $NH_2$- N $(CH_3)_2$ - N $(C_2H_5)2$ etc. It is prepared by the action of nitrous acid, with excess of m phenylnediamine). It consists of a mixture of hydrochlorides of mono and bisazo derivatives (Finar 1957)

**MATERIAL AND METHOD**

**Diluent used:**

The treatment of the toxicants to the fishes were given by bath. The fish were kept in the water containing the toxicants. The water used for dissolving the toxicants was supplied by the .overhead tank of P.G.Department of Zoology, Hindu College, Moradabad. The Physio mount that can be considerably toxic to chemical properties of the diluting water (Table-l) were analysed by the standard method given by APHA *et.al*.. (1976).

## Physio – Chemical characteristics of water

| Characteristics | Value (Ranges) |
|---|---|
| pH* | 6.2 -7.4 |
| Alkalinity | 25 – 62 |
| Dissolved oxygen (Do) | 5.4 – 7.9 |
| Total Solids | 26.5 – 33.8 |
| Organic Nitrogen | 11.3- 13.7 |
| Sulphates | 4.7 - 6.9 |
| Volatile solids | 13.3 -14.2 |
| Chlorides | 1.4 – 1.8 |

’Values except pH are expressed in mg/1.

**COLLECTION OF SPECIMEN:**

Live fishes were collected from the local fresh water resources such as ponds

and river. The fishes were transferred to glass aquaria for acclimatisation for a minimum period of one weak. Before transferring the fishes to the aquaria the fishes were washed by 0.1% potassium permanganate solution as to make the specimen devoid of ectoparasites.

**BIOASSAY DETERMINATION:**

Acute toxicity test was performed to determine the toxicity of the toxicants for *Labeo rohita*. By this test a wide range was determined and in this wide range lay the concentration that caused 0.00 to 100.0%nortality. By doing this the number of concentration to be tested were reduced. Thereafter atleast ten individuals were taken for each definite toxicity test and were exposed to each concentration of the toxicants.

Basically acute toxicity test follows four designs

l. Static

2. Static with renewal

3. Continuous or intermittent flow

4. In .situ. with an increasing order complexity.

Considering all the advantages and disadvantages, static with renewal design was found to be more suitable under the prevaient conditions. The dilution technique was adopted to get the required concentration of both the toxicants for the fish. The measured quantity. (by Hit and trial) 35 to 190 mg/l of Metanil yellow and 35 to 170 mg/l of Bismark brown for *Labeo rohita* were added in separate aquaria and was diluted by water to a required level. The quantity of water was 1 litre per fish.

After diluting the toxicants in the aquaria the fishes were transferred to there aquaria. taking all the precautions such as avoiding any physical stress during transfer. The aquaria were under careful observation all through and any dead fish was removed immediately from the aqueria to prevent the loss of dissolved oxygen in water. The survival percentage of the fish was calculated at 96 hrs. exposure . TLm (median tolerance limit) for both toxicants were calculated by graphical interpolation method. (APHA .*et.al.* (l985).concentration in log mg/,l, (upto 4 places by table given by Fisher and Yates 1963) were taken on Y-axis and the survival percentage on X-axis. The TLm was

directly read out from the graph against 50% survivality or mortality.

**Selection of doses and treatment:**

From the curves plotted for *Labeo rohita* .two sub lethal concentrations (one each for acute and chronic exposure for both the dyes) more suitable under the prevalent conditions. were selected. which gave 10-30% mortality and at which 70-90% fishes were able to survive after 96 hrs. in case of acute exposure and after 30 days of chronic exposure

respectively.

In case of acute exposure the concentration for Metanil yellow and Bismark brown is 55-mg/l and 50 ml/l for *Labeo rohita* after 96 hrs. of exposure respectively.

In case of chronic exposure the concentration for Metanil yellow and Bismark brown is 33 mg/l and 36 mg/l for *Labeo rohita* after 30 days respectively.

The fishes were divided into three batches for each toxicant and at each exposure. Each batch contained 80 healthy fishes. Two batches were treated by the toxicant with above mentioned concentration and one hatch served as control.

The fishes of the third batch was kept in ordinary tap water under identical physio-chemical conditions. The temperature range during the experiment was 18°C $\pm$ 4°C. The fishes in the ordinary tap water served as control as compared to Metanil yellow and Bismark brown. respectively. All the fishes were fed with fish food, suji and dhuta every alternate day and some times on the fourth day The bathing solution was changed every alternate day to avoid the impact of excreta. During experimentation the aquaria were covered with black paper to avoid any possible photo-oxidation of the dye. The observation for each experimental time were recorded after 48 hrs., 96 hrs.. 15 days and 30 days.

**RESULT**

The dilution technique was adopted to find out the required concentration of the toxicants. The measured quantity of both the dyes (Metanil yellow' and Bismark brown) were separately added to different glass aquaria and then diluted upto the required level.

Prior to the treatment fishes were washed with 0.1% potassium per manganate solution to avoid any possible pathogen from the skin. The fishes were then transferred to the experimental containers to different concentrations of toxicants from acclimatizing tank with the help of a small net. During this experiment precautions were taken as suggested in 'Standard Method' (APHA *et.al*. 1985;)‘ After every 24 hrs. the pH of the experimental solution was checked and was maintained to a constant pH (pH 6.8 to 7.4). The quantity of water evaporated was compensated every day. During experiments the behaviour of the fish was observed carefully . The dead fish if any were removed immediately so that such mortality may not deplete dissolved oxygen in the container during the bioassay test.

The median tolerance limit (TLm) or lethal concentration was calculatad for 96 hrs. by interpolating the data on semi logarithmic co-ordinate paper with concentration on the Y axis and survival percentage on X axis. A straight line between two points representing survival at two concentration that were lethal to more then half and less than half of the fishes, taken. The concentration at which the curve crossed 50% survival line represented median tolerance limit (T.L.m) or 50% lethal concentration value.

The TLm or 50% lethal concentration for *Labeo rohita* was calculated to be 2.0414 Log mg/l for Metanil yellow and 2.000 Log mg/l for Bismark brown.

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# L.C. 50 VALUE OF LABEO ROHITA FOR 96 HRS. EXPOSURE OF METANIL YELLOW

| CONCENTRATION Mg/1 | CONCENTRATION Log Mg/1 | SURVIVAL % | MORTALITY % |
|---|---|---|---|
| 35 | 1.5441 | 100 | 00 |
| 40 | 1.6021 | 95 | 05 |
| 45 | 1.6532 | 90 | 10 |
| 50 | 1.6990 | 85 | 15 |
| 55 | 1.7404 | 80 | 20 |
| 60 | 1.7782 | 75 | 30 |
| 65 | 1.8119 | 70 | 30 |
| 70 | 1.8451 | 65 | 35 |
| 75 | 1.8751 | 65 | 35 |
| 80 | 1.9031 | 60 | 40 |
| 85 | 1.9094 | 60 | 40 |
| 90 | 1.9542 | 55 | 45 |
| 100 | 2.0000 | 55 | 45 |
| 110 | 2.0414 | 50 | 50 |
| 120 | 2.0792 | 40 | 60 |
| 130 | 2.1139 | 35 | 65 |
| 140 | 2.1461 | 30 | 70 |
| 150 | 2.1761 | 25 | 75 |
| 160 | 2.2041 | 20 | 80 |
| 170 | 2.2304 | 10 | 90 |
| 180 | 2.2553 | 05 | 95 |
| 190 | 2.2788 | 00 | 100 |

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## L.C. 50 VALUE OF LABEO ROHITA FOR 96 HRS. EXPOSURE OF BISMARK BROWN.

| CONCENTRATION Mg/1 | CONCENTRATION Log Mg/1 | SURVIVAL % | MORTALITY % |
|---|---|---|---|
| 35 | 1.5441 | 100 | 00 |
| 40 | 1.6021 | 95 | 05 |
| 45 | 1.6532 | 90 | 10 |
| 50 | 1.6990 | 85 | 20 |
| 55 | 1.7404 | 80 | 20 |
| 60 | 1.7782 | 75 | 25 |
| 65 | 1.8119 | 70 | 30 |
| 70 | 1.8451 | 70 | 30 |
| 75 | 1.8751 | 65 | 35 |
| 80 | 1.9031 | 60 | 40 |
| 85 | 1.9094 | 60 | 40 |
| 90 | 1.9542 | 55 | 45 |
| 100 | 2.0000 | 50 | 50 |
| 110 | 2.0414 | 45 | 55 |
| 120 | 2.0792 | 35 | 65 |
| 130 | 2.1139 | 30 | 70 |
| 140 | 2.1461 | 20 | 80 |
| 150 | 2.1761 | 10 | 90 |
| 160 | 2.2041 | 05 | 95 |
| 170 | 2.2304 | 00 | 100 |

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TABLE NO. 9.2

SUMMARY OF EXPERIMENTS WITH LABEO ROHITA

| S.NO. | Name of Dye | No. of Fishes | Weight (gm.) | Temperature °C | Concentration mg/l | Duration of treatment | No. of Fishes died | No. of Fishes Survived | BEHAVIOUR |
|---|---|---|---|---|---|---|---|---|---|
| 1. | 4'AASA | 10 | 25 | 18±4 | 190 | 48hrs. | All | Nill | The dose proved to be lethal. The fishes started jumping over water followed by the lost balance, erractic movement for some time and after 48 hrs. died. |
| | DAAB | 10 | 25 | 18±4 | 170 | 48hrs. | All | Nill | |
| 2. | 4'AASA | 10 | 25 | 18±4 | 110 | 96hrs. | 5 | 5 | Initially there was jumping over surface of water followed by somersalts movement and then normal movements were regained. |
| | DAAB | 10 | 25 | 18±4 | 100 | 96hrs. | 5 | 5 | |
| 3. | 4'AASA | 10 | 20 | 15±4 | 55 | 15days | 2 | 8 | Initially the fish started moving on one side but after some time normal movements were regained. |
| | DAAB | 10 | 20 | 15±4 | 50 | 15days | 2 | 8 | |
| 4. | 4'AASA | 10 | 20 | 15±4 | 35 | 30days | Nil | All | Normal movements were observed. |
| | DAAB | 10 | 20 | 15±4 | 35 | 30days | Nil | All | |

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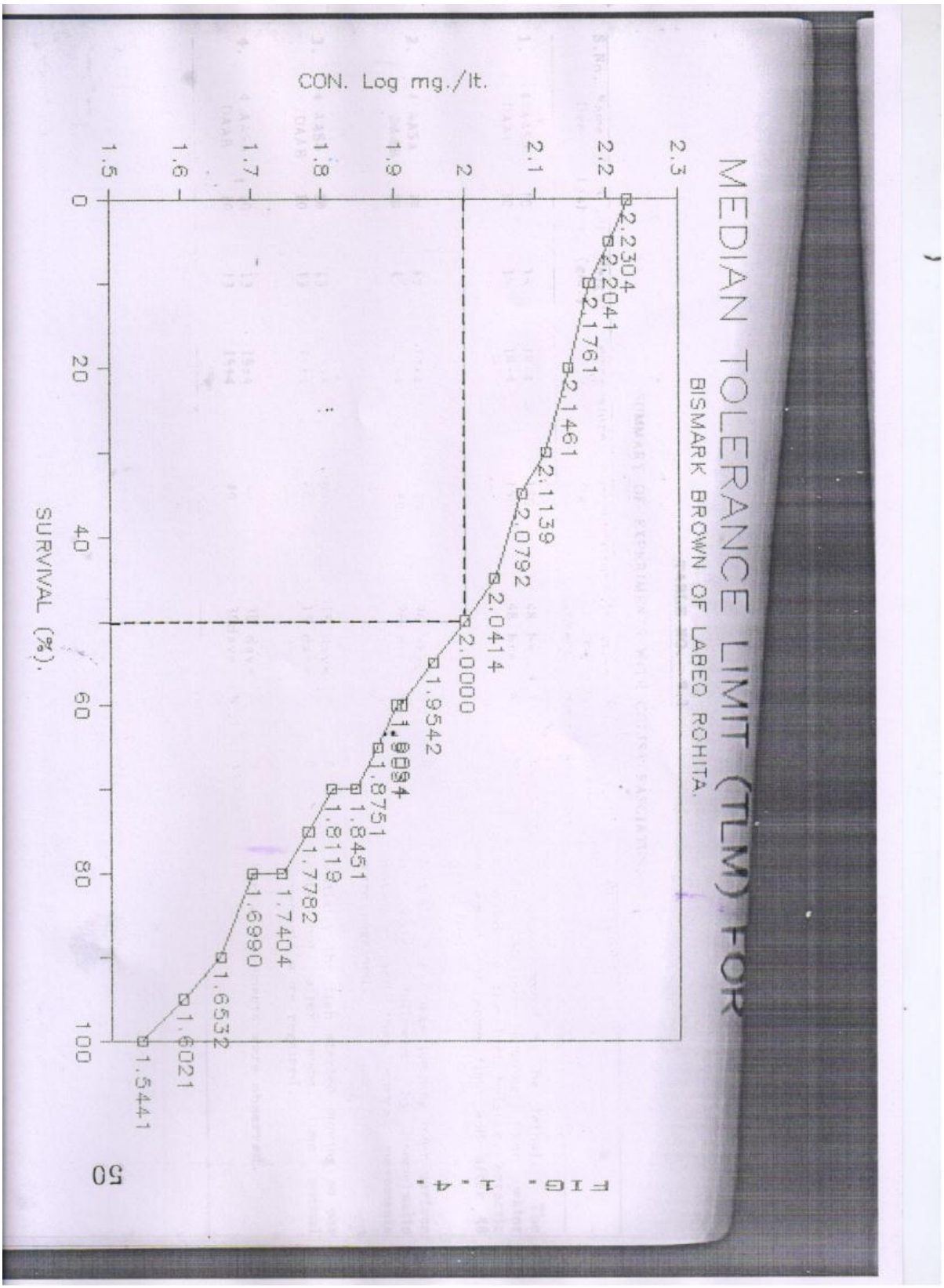

FIG. 4.4.

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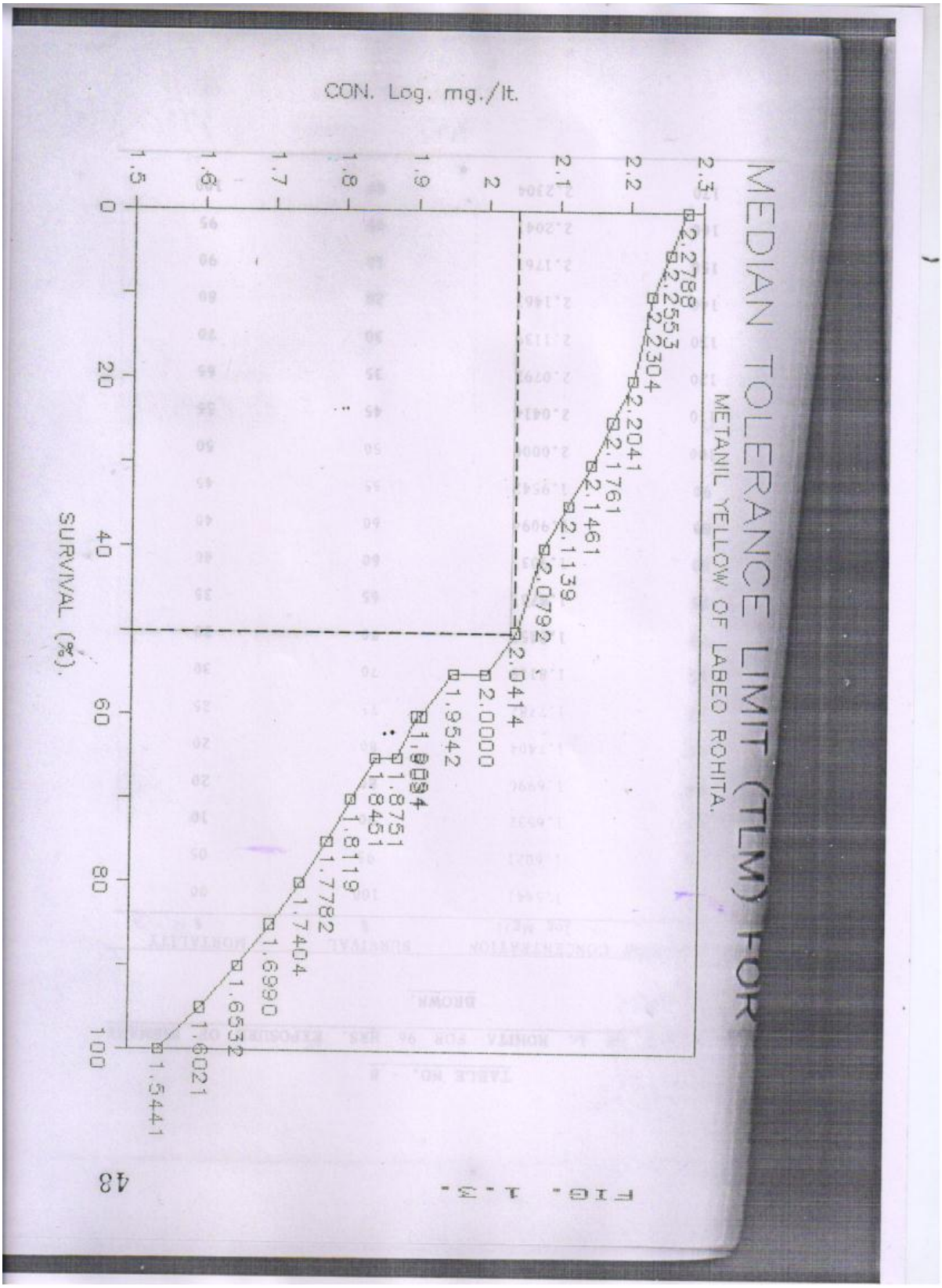


## DISCUSSION

Bioassay studies are often employed as a very first step in determining the toxicity

of a compound. In bioassay determination acute toxicity tests are considered to be most important. Since they provide information about the relative lethality of waste materials (Doudroff *et.al.* and Sprague ,1969 – 70 ). A number of workers ( Brungs and Mount, 1978 and Macek *et. al.* 1978 ) have evaluated various types of toxicity tests to assess effects of chemicals to aquatic life. According to Macek *et.al*. (1978) acute toxicity tests are ecologically significant, most scientifically and legally defensible, modest in predictive capability, most simple and of greatest utility.This is also being elaborated by APHA *et.al*. (1985). Buikema *et. al*. (1982) have suggested the application of exploratory test in acute tocity determination . The TLm recorded for *Labeo rohita* in case of metanil yellow and bismark brownwere 110mg/l and 100 mg/l respectively at 96 hrs. exposure.Singh and Sahai (1984b) have reported LC50 values 6ppm/l for *Rosbora daniconius* and 4ppm/l for *Puntius ticto* in 96 hrs. Exposure to malathion.

Maheshwari *et. al*. (1988) made observations on bioassay of three organic pesticides and found LC50 values to be 0.00224 mg/l, 0.0000569 mg/l and 0.0000502 mg/l for BHC, endosulfan and methylparathion for *H. Melitrix* respectively. Goel *et. al*. (1982c) and Sharma *et al* (1982) have noted 0.0175% of congo red to be LC50 for *Clarias batrachus* and *Heteropneustes fossili* respectively. Sharma and Gupta (1983) have reported 0.015% of direct deep black and 0.02% of chrysophenine – G LC50 for *Colisa fasciatus* at 96 hrs exposure. J. Selvanathan *et. al*. (2011) determined the LC50 values for cadmium chloride and mercuric chloride were 8.21 ppm and 1.85 ppm respectively for *Clarias batrachus*. Smt. Rani , K. (1999) found median lethal concentration of aluminium sulphate for 24 hrs. was 44.90 ppm and for 96 hrs was 26.42 ppm for a fresh water fish *Cyprinus carpio*. Manoj Kumar et. al. (2007) evaluated the LC50 value to be 0.48 ml/l, 0.28 ml/l, 0.18 ml/l and 0.03

ml/l for 24, 48 , 72 and 96 hrs for *Heteropneustes fossilis* under the stress of linear alkyle benzene sulphate.LC50 value of atrazine for *Channa punctatus* was determined as 42.381 $mgL^{-1}$ at 96 hrs exposure by Christopher Ddidigu et. al. (2010).Ravindran and Radhakrishnan (2015) determined LC50 of vanadium to the fresh water fish *Hetropneustes fossilis* to be 70.56 ppm, 68.15ppm, 66.60 ppm and 65.50 for 24, 48, 72 and 96 hrs exposure. Muthukumaravel *et. al.* (2013) reported the LC50 value at 24, 48, 72 and 96 hrs exposure of

monocrotophos were 0.0041, 0.0039, 0.0037 and 0.0036 respectively. LC50 value for Lambda cyhalothrin for 24, 48, 72 and 96 hrs were 0.0026, 0.0024, 0.0022 and 0.0021 respectively for *Labeo rohita*. Johnson and Radhakrishnan (2015) observed 40.56 ppm, 38.15 ppm, 36.65 ppm. And 35.50ppm at 24, 48, 72 and 96 hrs exposure in *Clarias batrachus* for chromium. Swetha et.al. (2015) reported LC50 for deltamethrin acute ( 24 and 96 hrs) were found to be 0.438 and 0.38 $mgL^{-1}$ in *Labeo rohita*. Thangan and Veeramani (2013) found the

median lethal concertration of platinum to Indian major carp *Cirrhinus mrigala* for 24 hrs and 96 hrs to be 12mg/L and 6mg/L.

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