3.1 INTRODUCTION

The effects of pesticides on aquatic fauna, particularly fishes, may be exhibited in a variety of ways, since the majority are non-selective and produce detrimental and sometimes fatal side effects on non-target species. Studies on the sublethal effects of pesticides have gained a great deal of impetus in the last decade, partly because of their practical importance and partly due to academic interest. Quantitative assessment of the effects of pollutants has got cardinal importance in any pollution research, both from the biological and ecological points of view.

There are various ways of investigating sublethal effects, and each technique provides an insight into the physiology or behaviour of the organism in question (Waldichuk, 1979). Efforts were made to evaluate the lethal and sublethal effects of commercial grade pesticides individually on a selected non-target vertebrate. The animal used in the study was the Asian cichlid fish *Etroplus maculatus* commonly called the orange chromide and has both freshwater and estuarine distribution.
3.2 TEST ANIMAL

*Etroplus maculatus* (Bloch)

*Etroplus maculatus* is the smaller of the two species representing the cichlidae family in India and is indigenous to South India and Sri Lanka (Munro, 1955). *E. maculatus* is a euryhaline fish sexually monomorphic, having an yellow ground colour with black markings and very common in the rivers, ponds, paddy fields, canals, lakes and estuaries of Kerala. This fish attains a maximum length of 6-8 cms and its small size considerably limits its utility as a food fish, however, it yields a minor fishery of economic value in certain parts of South India, particularly in the coastal areas of Kerala (Alikunhi, 1947).

*E. maculatus* is a laboratory favourite of ethologists. There are studies on its behavioural ontogeny (Wyman and Ward, 1973), courtship behaviour (Barlow, 1970), parent-offspring communication (Cole and Ward, 1970), aggressive behaviour (Reyer, 1975) and reproductive colouration (Rechten, 1980). The ecological importance of *E. maculatus* was highlighted by Wyman and Ward (1972) as a cleaning symbiosis exists between *E. maculatus* as cleaner and *E. suratensis* as the host. The young of *E. maculatus* actively cleans all age groups of *E. suratensis* which is of high economic importance and the cleaning
activity shows a daily circadian rhythm. The removal of fungus from the fins and tail appears to be an important adaptive function of this symbiosis.

Live specimens of *Etroplus maculatus* for the study were collected from the shallow inland water areas confluent with Cochin backwaters. The animals were collected using castnets causing minimum stress, and then transported to the laboratory in oxygen packs.

3.3 LABORATORY PROCEDURES

3.3.1 Laboratory conditioning of test animal.

The animals transported to the laboratory were maintained in large fibre glass tanks of 150 litre capacity containing well aerated water of corresponding salinity of collection areas (7.5 ± 7.5‰). They were acclimated for one week and observations were made on mortality, disease symptoms or abnormal behaviour of fishes, if any. The lots showing more than 5% mortality were discarded. During the acclimation period, salinity was gradually reduced to zero and the animals were maintained at 10‰ salinity for further 48 hours before the commencement of the experiments and were fed with minced clam meat and earthworm pieces. All organisms used for any one set of experiment belonged to the same population. Only healthy and adult animals of
the same size (6 ± 1 cms. in length group) were used for experiments, irrespective of sex.

The test medium used for the study was collected from Cochin backwaters, kept for aging in dark, filtered, diluted to zero salinity with dechlorinated tap water and aerated to full saturation before use. The pH of the experimental water was 7.5 ± 0.5. The addition of toxicants did not bring about any appreciable variation in pH. All the experiments were carried out at laboratory temperature (30 ± 1.5°C).

3.3.2 Toxicants.

The toxicants selected were the commercial formulations of three widely used pesticides, namely DDT, Dimecron and Gramoxone belonging to organochlorine, organophosphate and bipyridylium compounds, respectively. These three groups of compounds are being used extensively in agricultural and horticultural practices.

The pesticide solutions were prepared separately and added to the test media to get the desired concentrations. The DDT concentrations were prepared by mixing commercial formulation with acetone as vehicle solution in 1:1 ratio. Dimecron and Gramoxone are water soluble and the stock solutions were prepared in distilled water.
3.3.2.1 DDT\textsuperscript{R} 25% EC

DDT\textsuperscript{R} 25% EC studied is an emulsion concentrate containing 25% (w|w) DDT technical (1,1,1-Trichloro-2,2-bis-P-chlorophenyl ethane) and marketed by Premier Pesticides (P) Ltd. Though banned in agricultural operations, it is widely used in public health service for the control of mosquitoes.

3.3.2.2 Dimecron\textsuperscript{R}

Dimecron\textsuperscript{R} used for the study is the product of Hindustan Ciba-Geigy Ltd. It is a vinyl phosphate insecticide and the commercial formulation used contain 85% (w|w) of the active ingredient phosphmidon technical (1-chloro-1-N,N-diethyl carbamoyl-1-pzopenyl-2-dimethyl phosphate), and is water soluble.

3.3.2.3 Gramoxone\textsuperscript{R}

Gramoxone\textsuperscript{R} is manufactured by IEL Ltd. and is a trade mark of the Imperial Chemical Industries Ltd. PLC, London. The commercial formulation contains 24% (w|w) of the active ingredient, paraquat dichloride (1,1'-dimethyl-4,4'-bipyridium dichloride), and is water soluble. Gramoxone is mainly used as an agricultural and horticultural pesticide and is a potent inhibitor of photosynthesis in plants.
3.3.2.4 Toxicant concentration

The various concentrations of the toxicants are expressed in ppm in terms of the individual pesticide formulation. Commercial formulations were used and calculated quantity was weighed out to give the desired concentrations in the test medium.

3.3.3 Toxicity studies.

3.3.3.1 Lethal toxicity of individual toxicants

Lethal toxicity studies provide information about the relative lethality of a toxicant. This test is designed to determine the highest concentration of a pollutant that is sufficient to affect some percentage, usually 50% of a limited number of organisms. Though lethality appears to be a crude method of measurement of toxic response, its importance was highlighted by many workers (Duke, 1974; Buikema Jr. et al., 1982).

The static renewal test technique, as described by the American Society for Testing and Materials (1980), and the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975), was employed for the current study. Exploratory tests were conducted before performing full scale acute toxicity tests.
Experiments were carried out to assess the individual lethal toxic responses to the three pesticides, DDT, Dimecron and Gramoxone by the fish *Etroplus maculatus*. Laboratory conditioned fishes of uniform size (6 ± 1 cms in length) were exposed to 50 litres of test solution that contained graded, logarithmic series of concentrations of the toxicants. Fibre glass tanks, inner coated with chemical resistant epoxy resin, were used for the toxicant exposure. Ten animals were used for each test concentration of the toxicant. The experimental tanks were kept covered to minimize external disturbances. The tests were carried out at room temperature (30°C ± 1.5°C) and the animals were not fed during the test period. Appropriate duplicates and controls were invariably maintained for all the experiments. The test media were replenished totally every 24 hour. The animals were inspected at regular intervals, and were considered dead if it did not respond to mechanical stimulation, and the opercular movements ceased. The dead animals were removed and the cumulative percentage mortality at every 12 hour recorded. The LC 50 values and their 95% confidence limits were calculated as mentioned in section 3.4.
3.3.3.2 Short term sublethal toxicity studies

The objective of these toxicity tests was to find out the concentrations of the toxicants capable of inducing abnormal responses as well as the nature of the responses of some selected physiological and haematological indices of the test animals. The details of the selected physiological and haematological parameters are mentioned in sections 3.3.4 and 3.3.5.

The sublethal concentrations of different toxicants employed for the studies were computed in relation to the 96 h LC 50 value of the individual toxicants delineated after static bioassay studies. In the present study 1/2 of the individual 96 h LC 50 values were taken as the highest concentration along with three other concentrations fixed in the descending order. The duration of these experiments extended upto 120 hours, and the assessment of the selected parameters was carried out in animals pre-exposed to different sublethal concentrations for 24, 72 and 120 hours to the pesticides. Animals were not fed during short term studies.

3.3.3.3 Long term sublethal toxicity studies

A study of the toxicity after prolonged exposure of the test organisms to toxicants is a recent development in pollution experimentation. As in short term studies, the concentrations were selected
in relation to 96 h LC 50 value but the concentrations employed were very low. In the present study 1/10th of the individual 96 h LC 50 values were taken as the highest concentration along with three other lower concentrations fixed in the descending order. The test media were changed daily with fresh ones and the animals were fed with clam meat and pieces of earthworm, on alternate days, for 1–2 hours before the replenishment of the test media. The parameters studied were the same as in short term studies, and the evaluations were carried out in pre-exposed animals at 10, 20 and 30 days to the three pesticides.

3.3.4 Estimation of InVivo enzymatic activity

3.3.4.1 Preparation of enzyme extract

The control as well as the experimental fishes were sacrificed at the end of each test period (section 3.3.3.2 and 3.3.3.3) and the brain, gill and liver tissues were removed immediately. After rinsing in chilled glass double distilled water, accurately weighed, pooled tissues were homogenized separately in 0.25 M sucrose solution using a potter-Elvehjem type homogenizer. A 5% wet homogenate of the three tissues were prepared in the present study. The supernatent, obtained after centrifugation at 20,000 rpm at 4°C for 15 min, was the
source of the selected enzymes and for protein estimation during the present investigation. The supernatent was kept frozen and analysed for the enzyme activity within 2-3 hours after preparation. Care was taken to maintain the tissue and extract chilled till incubation.

3.3.4.2 Assay of Alkaline phosphatase

EC 3.1.3.1 (orthophosphoric monoester phosphohydrolase, alkaline optimum)

The same procedure as described in section 3.3.4.3 was adopted to estimate the activity of alkaline phosphatase with the following changes. Instead of citrate buffer, 0.27 Glycine—sodium hydroxide buffer of pH 9.2 was used. NaCl and MgCl₂ were added to the buffer to get a concentration of 100 mg NaCl and 0.1 mg of MgCl₂ per 1 ml of the buffer. The volume of the extract used was 0.1 ml and 2 ml of 0.25 N NaCl was used to stop the reaction. The calculations and the unit of enzyme activity were the same as described in section 3.3.4.3.

3.3.4.3 Assay of Acid phosphatase activity (EC 3.1.3.2)

Acid phosphatase (orthophosphoric monoester phosphohydrolase, acid optimum) activity was determined following the methodology described in Sigma Technical Bulletin No.104 (9-82) with slight modifications. To 2 ml of 0.1 M frozen citrate buffer of pH 5.3 containing
100 mM NaCl, 0.1 ml of the enzyme extract was added. The buffer–enzyme mixture was incubated in a thermo-controlled water bath at 37 ± 0.05°C and to this reaction mixture 0.1 ml of the substrate (2 mgs of P-Nitrophenyl phosphate sodium salt [Merck] in 0.1 ml glass double distilled water) was added to initiate the reaction. After 1 hour incubation at 37°C, 4 ml of 0.25 N NaOH was added to the buffer–enzyme substrate reaction mixture to stop the activity of the enzyme. P-Nitrophenol formed during incubation by the hydrolysis of P-Nitrophenyl phosphate, catalyzed by acid phosphatase, gives an yellow colour in alkaline pH and the colour was read spectrophotometrically at 410 nm. The concentration of P-Nitrophenol formed was calculated from the standard graph. Simultaneously, the protein content of the extract was estimated by Lowry’s method (1951). From this μ mol of P-Nitrophenol liberated per milligram protein per hour was calculated and the enzyme specific activity is expressed as μ mol of P-Nitrophenol liberated/mg protein/hour.

3.3.4.4 Assay of Glutamate Oxaloacetic Transaminase (GOT) or Aspartate Amino Transferase AsAT (EC 2.6.1.1)

The estimation of GOT activity was carried out by the caloriometric method of Reitman and Frankel (1957) as described in Methods of Enzymatic Analysis (1974). For estimating GOT, phosphate buffer|
substrate of pH 7.4 containing 0.1 M phosphate buffer, 0.1 M aspartic acid sodium salt, and 2 mM 2-oxoglutarate was used. The buffer-substrate mixture containing 0.1 ml of enzyme extract was incubated at 37°C for one hour. At the end of incubation the enzymatic reaction was stopped by adding 1 ml of 1 mM chromogen in HCl (2,4-dinitrophenyl hydrazine) mixed well and kept for 20 minutes at room temperature. After 20 minutes, the reaction mixture was made alkaline by adding 10 ml of 0.4 N NaOH. The colour developed by 2,4-dinitrophenyl hydrazone of the reaction product, pyruvate was determined spectrophotometrically at 546 nm. Sodium pyruvate was used to prepare the calibration curve. The estimation of protein was done by Lowry's method (1951).

3.3.4.5 Assay of Glutamate Pyruvate Transaminase (GPT) or Alanine Amino Transferase (AlAT) (EC 2.6.1.2)

Colorimetric method of Reitman and Frankel (1957) was adopted for the estimation of GPT and the procedure was the same as that for GOT with following changes. The buffer-substrate solution contained 0.1 M phosphate buffer of pH 7.4, 0.2 M DL-alanine (instead of aspartate) and 2 mM 2-oxoglutarate. The oxaloacetate formed during the reaction combined with chromogen to form 2,4-dinitrophenyl hydrazone of oxaloacetate which was read spectrophotometrically at 546 nm. Sodium pyruvate was used to prepare the calibration curve. Protein content of the extract was determined by Lowry's method (1951).
3.3.5 In vitro enzyme activity studies

For the in vitro enzyme activity studies only the enzyme extract (section 3.3.4.1) of the above said tissues of unexposed fishes were used. The desired concentrations of the individual pesticides were directly added to the buffer/substrate medium prior to enzyme extract addition. The procedure of the estimation of activity of the individual enzyme was the same as described for the InVivo studies (3.3.4.2).

3.3.6 Haematological Analysis

3.3.6.1 Collection of blood samples

Blood samples were collected from the caudal vein in aseptic condition by severing the caudal peduncle. With fish less than six inches in length, severance of caudal peduncle proved most feasible (Hesser, 1960). The blood samples collected in small glass vials were treated with 3:2 mixture of ammonium oxalate and potassium oxalate at the rate of 0.5-1 mg per ml of blood to prevent coagulation. Aliquots of pooled blood samples of 5 to 7 fishes was used for different estimations.
The different haematological analyses were carried out employing standard techniques (Hesser, 1960; Blaxhall and Daisley, 1973) unless specified.

3.3.6.2 Total Erythrocyte Count (TEC)

The techniques employed for the erythrocyte counts of fish blood were similar in most respects to those used in mammalian counts except a change in RBC diluting fluid. Hendrick's RBC diluting fluid was used during the present study (Hendricks, 1952). The Hendricks fluid contained 10 gm of sodium sulphate, 2.5 gm of sodium chloride, 1.5 gm of sodium citrate and 50 ml of glacial acetic acid per 500 ml of distilled water. Neubauer type haemocytometer was used for RBC counting. Total erythrocyte count is expressed in millions of RBC per cubic mm of blood.

3.3.6.3 Estimation of Haemoglobin

Cyanomethaemoglobin method described by Ortho Diagnostic Systems (1986) was followed for estimating the haemoglobin content. To 0.02 ml of blood, 5 ml of Aculute reagent (modified Drabkin reagent) was added and stirred well. The potassium ferricyanide present in the reagent converts the haemoglobin iron from ferrous to ferric state to
form methaemoglobin and this in turn combines with potassium cyanide of the Aculute reagent to produce a stable pigment or the cyanomethaemoglobin which represents the sum of oxyhaemoglobin, carboxyhaemoglobin and methaemoglobin. The cyanomethaemoglobin formed was measured spectrophotometrically at 540 nm. The calibration curve was prepared by the Human Haemoglobin Standard provided with the Aculute reagent. The haemoglobin content is expressed as g% (or gm|dl).

3.3.6.4 Measurement of Haematocrit values
(or packed cell volume – Ht%)

Haematocrit value was measured by applying the method of McLeay and Gordon (1977). Blood was drawn into heparinised microhaematocrit tube (0.55 ± 0.05 mm diameter). One end of the tube was sealed and centrifuged in microhaematocrit centrifuge at 11500 rpm for 5 minutes. Haematocrit value was measured within 30 minutes of centrifugation and measured the red cell column using haematocrit counter provided along with the microhaematocrit centrifuge, and expressed as the percentage of whole blood.

3.3.6.5 Computation of Erythrocyte constants

From the values of Hb content (Hb%) haematocrit (Ht%) and total erythrocyte count (millions/mm³) the following erythrocyte
constants were calculated using the respective formula (Lamberg and Rothstein, 1978).

3.3.6.5.1 Mean Corpuscular Volume (MCV)

MC represents the average volume of individual erythrocytes in cubic microns ($\mu^3$) and computed by the formula:

$$MCV = \frac{Ht\%}{RBC \text{ (in million mm}^3\text{)}} \times 10$$

3.3.6.5.2 Mean Corpuscular Haemoglobin (MCH)

MCH represents the average weight of haemoglobin in individual erythrocytes in picograms (pg) and calculated by the formula:

$$MCH = \frac{Hb\%}{RBC \text{ (in million mm}^3\text{)}} \times 10$$

3.3.6.5.3 Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC is the average haemoglobin concentration per 100 ml of packed erythrocytes in percent and computed by:

$$MCHC = \frac{Hb\%}{Ht\%} \times 100$$
3.3.6.6 Calculation of Erythrocyte indices

From the values of TEC, Hb%, Ht%, MCV, MCH and MCHC the following erythrocyte indices were calculated using the respective formula. The erythrocyte indices of the control fish is taken as one and referred as unity in the text (Lamberg and Rothstein, 1978).

3.3.6.6.1 Volume index (VI)

Volume index is the ratio of the size of the erythrocyte of the experimental fish to that of the normal or control and computed by

\[
VI = \frac{\text{MCV of experimental fish}}{\text{Mean MCV of control fish}}
\]

3.3.6.6.2 Colour index (CI)

Colour index represents the amount of Hb in each erythrocyte compared with normal content and calculated by

\[
CI = \frac{\text{Mean RBC of control} \times \text{Hb of experimental fish}}{\text{Mean Hb of control} \times \text{RBC of experimental fish}}
\]
3.3.6.6.3 Saturation index (SI)

Saturation index is the degree of saturation of erythrocyte with Hb of experimental fish in relation to that of the normal subject and computed by the formula:

\[ \text{SI} = \frac{\text{MCHC of experimental fish}}{\text{Mean MCHC of control fish}} \]

3.3.7 Histopathology

There are no standardized techniques for examining tissues in aquatic organisms. However, standard medical and veterinary techniques may be modified and used to diagnose tissue changes in fishes. The fishes were exposed to the highest sublethal concentration of individual pesticides for 20-25 days (section 3.3.3.3). The histological techniques and staining procedures to prepare the tissue sections for microscopic examination were mainly adopted from the methods described by Bucke (1972) and Bullock (1978). At the end of the test period the brain, gill and liver tissues were dissected out and fixed in Bouin's fixative for 24 hours. After fixation the tissues were graded in ascending alcohol series and cleared in methyl-benzoate for 3 to 5 hours. The gill tissue was decalcified in 8% formic acid before alcohol grading. The methyl-benzoate cleared tissues were embedded in paraffin wax after proper paraffin infiltration. The sections were cut at 7 μ thickness using the rotary microtome.
After deparaffinisation in xylene, the sections were hydrated and stained with Ehrlich's hematoxylin for 2 to 5 minutes. Stained sections were then washed in running water for 3 to 5 minutes, dehydrated in graded alcohol, and counterstained with 95% alcoholic Eosin for 2 to 5 minutes. After further dehydration (in absolute alcohol) and clearing (in xylene), the sections were mounted in DPX.

3.4 COMPUTATION AND PRESENTATION OF DATA

The median lethal concentration (LC 50) levels and their 95% confidence limits were computed using the computer software developed by the Institute for Inland Water Management and Waste Water Treatment, Netherlands, based on probit analysis (Finney, 1957). The lethal toxicity experiments were repeated wherever necessary. The reliability of the LC 50 level was checked by the students' t test (to see any significant variation at 5% level between the experiments). If highly significant variation was observed, the experiment was repeated again. To report, LC 50 levels with least variance and/or lowest values were selected. The LC 50 levels, ET 50 values and toxicity curves were represented graphically to demonstrate the lethal effects of individual pesticides following approved methods (Sprague, 1973).
Graphical representation, together with Tables have been used to explain the experimental results on enzyme activity and haematological studies. These data have been subjected to statistical analysis using students 't' test to manifest the variation in comparison with the control. The variations were reported at three significant levels, viz. p <0.05, 0.01 and 0.001.

All the computations involved in the work were carried out by a personnel computer (HCL model -Busybee PC\|\XT).

3.5 TERMINOLOGY AND ABBREVIATIONS

The terminology, related to lethal toxicity studies, used in the present work are those adopted by Sprague (1969, 1970, 1971). The term median lethal concentration (LC 50) corresponds to LD 50, universally used in toxicology. Instead of the actual concentrations, abbreviations like C_1, C_2, C_3 and C_4 are used in the text to denote the four sublethal concentrations (SLC) of individual pesticide used for convenience of representation and the four SLC used in short term studies are different from that of long term studies (sections 3.3.3.2 and 3.3.3.3). The C_1 corresponds to the lowest exposed concentration and C_4 to the highest while C_2 and C_3 represents the two intermediate concentrations. The four SLC of individual pesticide used in short
Term and long term studies corresponding to $C_1$, $C_2$, $C_3$ and $C_4$ are listed below.

<table>
<thead>
<tr>
<th></th>
<th>Short term (ppm)</th>
<th>Long term (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DDT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_1$</td>
<td>0.00033</td>
<td>0.00013</td>
</tr>
<tr>
<td></td>
<td>(3.3-04)</td>
<td>(1.3-04)</td>
</tr>
<tr>
<td>$C_2$</td>
<td>0.00065</td>
<td>0.00017</td>
</tr>
<tr>
<td></td>
<td>(6.5-04)</td>
<td>(1.7-04)</td>
</tr>
<tr>
<td>$C_3$</td>
<td>0.0013</td>
<td>0.00026</td>
</tr>
<tr>
<td></td>
<td>(1.3-03)</td>
<td>(2.6-04)</td>
</tr>
<tr>
<td>$C_4$</td>
<td>0.0026</td>
<td>0.00052</td>
</tr>
<tr>
<td></td>
<td>(2.6-03)</td>
<td>(5.2-04)</td>
</tr>
<tr>
<td><strong>Dimecron</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_1$</td>
<td>0.01</td>
<td>0.0043</td>
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<td></td>
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</tr>
<tr>
<td>$C_2$</td>
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<td>0.0058</td>
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<td></td>
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</tr>
<tr>
<td>$C_3$</td>
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<td></td>
<td>(8.7-03)</td>
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</tr>
<tr>
<td>$C_4$</td>
<td>0.086</td>
<td>0.017</td>
</tr>
<tr>
<td>Gramoxone</td>
<td>Short term (ppm)</td>
<td>Long term (ppm)</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>C₁</td>
<td>0.0034</td>
<td>0.0013</td>
</tr>
<tr>
<td></td>
<td>(3.4-03)</td>
<td>(1.3-03)</td>
</tr>
<tr>
<td>C₂</td>
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<td>0.0018</td>
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<tr>
<td></td>
<td>(6.7-03)</td>
<td>(1.8-03)</td>
</tr>
<tr>
<td>C₃</td>
<td>0.013</td>
<td>0.0027</td>
</tr>
<tr>
<td></td>
<td>(2.7-03)</td>
<td></td>
</tr>
<tr>
<td>C₄</td>
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<td>0.0054</td>
</tr>
<tr>
<td></td>
<td>(5.4-03)</td>
<td></td>
</tr>
</tbody>
</table>

In InVitro enzyme activity studies, uniform concentrations were selected irrespective of the pesticides and of its 96 h LC 50s and abbreviated as C₁, C₂, C₃, C₄ and C₅. The actual concentration and its corresponding abbreviations are listed below.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Abbreviation</th>
</tr>
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<tbody>
<tr>
<td>10⁻⁸ ppm</td>
<td>C₁</td>
</tr>
<tr>
<td>10⁻⁷ ppm</td>
<td>C₂</td>
</tr>
<tr>
<td>10⁻⁶ ppm</td>
<td>C₃</td>
</tr>
<tr>
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<td>C₄</td>
</tr>
<tr>
<td>and</td>
<td>10⁻⁴ ppm</td>
</tr>
<tr>
<td></td>
<td>C₅</td>
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</table>