I. Material:

*Oziotelphusa senex senex* (Fabricus) is a fresh water field crab inhabits in the paddy fields in and around Anantapur and in many districts of Andhra Pradesh. It makes burrows in soft mud along the edges of paddy fields and irrigated canals and lives inside burrows. It is a semiterrestrial crab as it can live on land for longer periods and also lives partly immersed in water. Apart from their economical importance and great commercial value, the crabs are easily collected, easy to handle and to maintain in the laboratory conditions. Hence, this crab is chosen as ideal experimental material particularly in toxicological studies as it is known to have a great adaptability to laboratory conditions. The detailed classification of the animal is as follows:

**CLASSIFICATION:**

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Arthropoda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subphylum</td>
<td>Mandibulata</td>
</tr>
<tr>
<td>Class</td>
<td>Crustacea</td>
</tr>
<tr>
<td>Subclass</td>
<td>Malacostraca</td>
</tr>
<tr>
<td>Superorder</td>
<td>Eucarida</td>
</tr>
<tr>
<td>Order</td>
<td>Decapoda</td>
</tr>
</tbody>
</table>
Suborder - Reptantia

Section - Brachyura

Genus - Oziotelphusa

Species - senex

Variety _ senex

Biology of *O. senex senex*:

*Oziotelphusa* is a eurythermal (Pampapathi Rao, and Venkata Reddy, 1976) and it is euryhaline (Ramamurthy, 1962, 1965; Venkata Reddy, 1976) decapod crustacean generally inhabits the paddy fields and irrigating canals of south India. This crab is formerly known as *paratelphusa hydrodromus* (Herbst) is carnivores in diet and normally feeds on insects, worms etc. canabolims is also seen in this species i.e. eating on the young ones. It is strictly inhabitant of freshwater and is found neither in brackish water or sea-water. However, yet it has the unique capacity to tolerate even direct transfer to 100% seawater. It is described as “Poor man’s protein” as in South India people relish to eat this crab.

The following aspects have prompted to select this crab *O. senex senex* as an ideal experimental animal. These crabs are generally present in the paddy fields may be easily prone to the pesticides applied to the field crops by killing the harmful insects and pests. The utilization of
this crab *O. senex senex* as a source for chiefer protein in this part of the country may lead to the accumulation of compounds into the tissues of the crab. Hence, studies on the effect of pesticides especially on environmental impact and risk assessment in this crab *O senex senex* are very much warranted.

Besides these, by virtue of its carnivorous feeding habits, abundant availability, greater adaptability to the laboratory conditions and to toxicants, hence this crab *o. senex senex* is chosen as ideal experimental material in the present investigation.

II Pesticide selected:

The present investigation deals with physiological and biochemical adaptations of freshwater field crab *oziotelphusa senex senex* exposed to sublethal toxicity of fenvalerate which is a pyrethroid pesticide.

Technical grade fenvalerate (90%) was obtained from Bharat pulverising Mills private Ltd., Bombay, India.

Physico – chemical Properties of Fenvalerate

<table>
<thead>
<tr>
<th>Common name</th>
<th>Fenvalerate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial name</td>
<td>Fenvalerate 20% EC (Sumicidin)</td>
</tr>
</tbody>
</table>
Chemical name: α-cyano (3-phenoxyphenyl) methyl-4- chloroalpha-(1-methyl) Benzene acetate.

Structural Formula:

Empirical formula: \( \text{C}_{25} \text{H}_{22} \text{O}_3 \text{NCL} \)

Molecular Weight: 419.9

Appearance and odour: yellow to light brown oily liquid at 25°C with characteristic odour.

Boiling Point: 300° / 37 mm Hg

Vapour Pressure: 2.8 x 1.0\(^{-7}\) / mm Hg at 25° C

Viscosity: 39300 CP at 23° C
4320 CP at 39° C
400 CP at 59° C

Specific gravity: 1.170 at 23° C

Refractive index: 1.5655 at 21.5° C
Solubility in solvent: 450 gms/litre of acetone; xylene chloroform / mg in 1 litre of water at 20°C.

III GENERAL EXPERIMENTAL PROCEDURE:

Factors Influencing Pesticide Toxicity:

The flow of water:

Since significant difference has been reported in the toxicity of pesticides between static and flowing water (Burke and Ferguson, 1969), the experiments of this investigation were conducted in static water conditions as suggested by Doudoroff et al., (1951).

Aeration of water:

Hypoxic condition resulted due to pesticide exposure has been reported earlier (Mayes, 1977). Hence, during experimentation, the water in the aquaria containing normal and fenvalerate exposed animals was aerated periodically to prevent anoxia or hypoxia as suggested by Khorram and Knight (1977).

Physico-Chemical properties of water used for the experiment:

1. \( \text{pH} \) : 7.4 to 7.6
2. Salinity : 0.191g/lit.
3. Dissolved O$_2$ : 6 to 7 ml/lit.
4. Chlorinity : 0.111 mg/lit.
5. Sodium : 122 m. moles/lit.
6. Potassium : 30.5 m. moles/lit.
7. Calcium : 4.31 m. moles/lit.
8. CO$_2$ : 2.09 mg/lit.
9. O$_2$% saturation : 8.0

Other factors:

The physiological functions of the animals are known to be affected by various other factors like temperature, salinity, diurnal rhythms etc. Hence, all these factors were kept constant as far as possible throughout this investigation to nullify their effects.

IV. Maintenance of crabs – (Experimental animals):

The crabs were collected from the local paddy fields, Anantapur and brought to the laboratory and were stored in large glass aquaria partially immersed in Fresh water. The water in aquaria was changed daily so as to provide fresh water rich in oxygen. All the crabs were allowed to be adapted to the laboratory conditions for at least one week at room temperature $27^\circ C \pm 1^\circ C$ and they were exposed to natural
photoperiod. During this period, crabs were fed daily with frog muscles and earthworms etc. Only male individuals were used to avoid the influence of sex and injured crabs were avoided.

As the present investigation deals with the influence of body size on the toxicity of fenvalerate, two different sizes of crabs weighing small (6 ± 1g), and large (16 ± 1g) were taken.

**DETERMINATION OF LC50 VALUES (EVALUATION OF FENVALERATE TOXICITY)**

**Method:**

The static bioassay is followed for the evaluation of the pesticide toxicity, where the biological response of the animal was recorded in static water (Doudoroff et al., 1951), so that any change due to extraneous influences can be easily be nullified by maintaining suitable control on crabs in static water.

For LC₅₀ determination (Finney, 1964), eight different concentrations of fenvalerate like 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1 and 1.2 ppm were prepared for each of small and large size crabs. A stock solution of 1000 ppm (mg/ml) of fenvalerate was prepared in acetone, since acetone is not completely soluble in water. Moreover, acetone is found to be non-toxic to the animals (Pickering et al., 1962). From this stock
solution, required dilutions as given above were prepared with distill water. The experimental crabs of different sizes small (6 ± 1g) and large (16 ± 1g) were separated into 9 batches of 10 each. One batch each (small and large) was exposed to freshwater without fenvalerate, while the other 8 batches each of small and large size crabs were exposed to 8 different fenvalerate concentrations as mentioned above. The mortality of these small and large size crabs in the different concentrations were noted down at 96hrs exposure (the crabs were starved during this exposure period). Each experiment was repeated for 3 times in these selected concentrations of fenvalerate.

$LC_{50}$ values were calculated by graphical plots, where % mortality verses fenvalerate concentration and probit mortality verses fenvalerate concentration (Finney, 1964) was taken. For subsequent verification of $LC_{50}$ values, the equation of Dragstedt – Behrens as given by Carpenter (1975) was employed, where the crabs were exposed to log$^2$ concentrations of fenvalerate (0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1 and 1.2 ppm) for each of small and large size crabs for 96 hours. The %mortality was calculated from the cumulative mortality (tables 1 and 2). The $LC_{50}$ values obtained by graphical method can also be verified by Dragstedt Behren’s method as given by Carpenter (1975).
\[ \log Lc_{50} = \log A + \frac{50 - a}{b - a} \times \log 2 \]

Where,

A = Concentration of pesticide which kills 50% of the animals.

a = % mortality observed immediately below 50% mortality.

b = % mortality observed immediately above 50% mortality.

The mean and the standard deviation of LC\(_{50}\) values (ppm) obtained by three sources was found to be 0.84 ± 0.065 for small, 1.08 ± 0.024 for large size individuals respectively (table.4).

**DETERMINATION OF NOEL**

**Method**

No observed effect level (NOEL) is an experimentally determined dose at which there is no statistical or biological significant effect of a pesticide, fenvalerate for example in the present investigation. In determining NOEL, safe concentrations of fenvalerate like 0.01 ppm, 0.03 ppm and 0.05 ppm were chosen and the \(O_2\) consumption of this crab was measured daily upto 30-days besides in the control medium (freshwater with out fenvalerate) by using improved Winkler's method (Bashamohideen and Kunnemann, 1978). Detailed procedure is given in the subsequent chapters. As no significant changes were observed in \(O_2\)
consumption of this crab at 0.01 ppm, this tracer concentration is treated as the level for NOEL.

**PREPARATION OF SUBLETHAL CONCENTRATION OF FENVALERATE FOR SUBSEQUENT STUDIES OF THIS INVESTIGATION**

There is an increasing evidence and realisation that the effect of pesticides on the response of the crab other than the easily observable mortality effects must be taken into account in evaluating the complete ecological impact of the contaminatory substance. This is especially important, because distinct changes involving sequences of events in the crab in response to pesticide toxicity are likely to occur to sublethal exposure (Anderson, 1982; Bashamohideen, 1984). Therefore, the sublethal concentration of Fenvalerate was derived from LC50 values obtained earlier for further studies in this investigation.

In the present investigation, the LC50 values of fenvalerate obtained for small and large size individuals were found to be 0.84 ppm and 1.08 ppm respectively. Since, the sublethal concentration is suggested to be taken approximately 1/5 of LC50 values (Reed and Muenchi, 1938; Konar, 1969), which happens to be 0.2 ppm and is chosen as ideal sublethal concentration for both body sizes of the crab for the sake comparison with
an exposure period of 30-days for further studies in the present investigation.

FENVALERATE EXPOSURE – TIME COURSE OF $O_2$ CONSUMPTION OF THE WHOLE CRAB

The time course in the rate of $O_2$ consumption ($O_2$ml/g/hr) was studied separately in different body size individuals of $O$. senex senex namely small ($6 \pm 1g$) and large ($16 \pm 1g$) in order to assess the physiological and biochemical responses (the sequence of events) during sublethal (0.2ppm) exposure of fenvalerate for a period of 30-days. Six crabs were studied individually from each size group. The $O_2$ consumption was measured by the improved Winkler’s method as devised by Bashamohideen and Kunneman, 1978.

Method:

The experimental crab was introduced into the respiratory chamber. The crab was allowed to settle in the chamber for a period of 5 minutes. The initial water samples were collected into the Winkler bottles of about 50ml in volume. Half an hour after the introduction of the crab, the final samples were collected into the Winkler bottles. The dissolved oxygen content in these samples were estimated as given below. With the help of micro syringes, 0.25ml of 40% manganous chloride, 0.25ml of 10%
potassium hydroxide and 0.25ml of 3% dye, Leucobarbeline Blue-I were added respectively into the bottles containing initial and final samples separately, which were then closed and shaken well for a minute and the precipitate was allowed to settle. After 3 minutes, 1ml of 40% critic acid was added and the bottles were again shaken for a minute in order to dissolve the precipitate. After 5 minutes, a deep blue colour was developed. 5 ml of this blue colour solution was pipetted out into a 50ml standard flask and was made upto the mark with distilled water. The optical density of blue colour was read in specotronic-20 spectrophotometer at a wavelength of 54/78nm using Glass Cuvettes of 1 cm light path. The intensity of the blue colour is directly proportional to the amount of oxygen present in the sample. The calibration curve was prepared by using water samples of different oxygen concentrations (by bubbling N₂ into the water). The equation of the calibration and optical density at 578 nm was calculated by linear regression. A calibration factor of 6.607 was obtained in the present method. The difference in oxygen concentration between the initial and final sample gives the rate of oxygen consumption by the following formula. The rate of oxygen consumption is calculated by linear regression. The rate of oxygen consumption is calculated by using the following formula.
Oxygen consumption (O$_2$ml/g/hr) =

\[
\frac{[\text{Initial O.D.} - \text{Final O.D.} \times 6.607 \times 2]}{\text{Body weight}}.
\]

The reagents used for this experimentation were purchased from Mevck (Darmstadt) and the dye from Altman (Berlin). The solutions were prepared with oxygen free distilled water. In order to prevent the decomposition of the dye, amber coloured bottles were used and 0.3ml of 25% ammonium hydroxide per 100 ml solution of the dye was used. The time course of the rate of oxygen consumption during sublethal exposure of fenvalerate was measured as detailed below:

To begin with, the rate of O$_2$ consumption of each crab was measured in the natural fresh water medium over a period of 4 days at 24 hrs interval. On the 5-day, the crab was transferred into the freshwater containing sublethal (0.2ppm) concentration of fenvalerate and immediately after its transfer, its O$_2$ Consumption was measured. Thereafter, the crab was maintained in this new medium and its O$_2$ Consumption was measured continuously at 24-hrs interval for a period of 30-days. Each measurement of the rate of O$_2$ Consumption was made in triplicate and the mean value was taken into account. The experimental crabs were fed normally during the course of these experiments. The medium was replaced by a fresh medium every day in order to prevent the accumulation of excretory
products by the experimental crabs and the possible degradation of the pesticide leading to its decreased concentration.

**FENVALERATE EXPOSURE – HEART BEAT RATE**

**Method:**

To begin with, the crab was dissected out just by removing the carapace on the dorsal side and exposed the heart. The crab was then placed in the required experimental medium. It was allowed 10 mts time to overcome the initial shock and the heart beat rate was determined when the heart that becomes regular. The rate of heart beat is the time taken for 10 heart beats (in seconds) and was calculated by using the formula \(10/T\), where '10' shows the number of heart beats and “T” shows the time taken in seconds for 10 beats. The study was made for 3 times in each crab and the average was taken into consideration.

The rate of heart beat in small and large size individuals of *O. senex senex* was subjected sublethal (0.2 ppm) fenvalerate exposure periods like 24 hrs, 8-day, 16-day, 24-day and 30-day besides control medium (freshwater without fenvalerate). The day time, 9.30 A.M was fixed for recording the heart beat and this time was maintained constantly to avoid any chronobiological effects. From each size group, 6 crabs were studied individually. The mean values of the six measurements were taken
into account. The maximum % of suppression and the % recovery in the rate of heartbeat were calculated and subjected to statistical analysis.

**FENVALERATE EXPOSURE – CARBOHYDRATE METABOLISM**

Haemolymph sugars, Hepatopancratic and Muscle glycogen levels were estimated separately in each size individuals of *O. senex senex* at different sublethal exposure periods of fenvalerate.

**Estimation of Haemolymph sugars:**

**Method:**

The level of haemolymph sugars from each size group individuals of *O. senex senex* subjected to different sublethal (0.2 ppm) fenvalerate exposure periods like 24hrs, 8-day, 16-day, 24-day and 30-day in relation to control medium (freshwater without fenvalerate) were estimated by the calorimetric method as described by Nelson Somagyi (1952). Crabs of the same size were taken and divided into 6 batches of 6 animals each. The haemolymph from the crabs was drawn by a hypodermal syringe through the arthrodial membrane of the chelate leg using 3% sodium oxalate solution as anti-coagulant. The haemolymph was then collected into the test-tubes. 0.2 ml of haemolymph was taken and to it were added 1.8 ml of deprotenizing solution (Zinc sulphate and sodium hyproxide solution in 1 : 1 ratio). The mixture was then centrifused at 3000
rpm for about 10 minutes. 1.0 ml of the supernatant was drawn and to it was added 1.0 ml of alkaline copper reagent. The mixture was then cooled under running tap water. Finally, 1.0 ml arsenomolybdate colour reagent was added and made up to 10 ml with distilled water. The optical density of the colour developed was measured in spectrophotometer at a wavelength of 540 nm. A blank and glucose standards were also run similarly. The level of haemolymph sugars in crabs were expressed as mg % (mg of sugars per 100 ml of haemolymph). The mean values of six measurements were taken into account.

Estimation of Glycogen content in Hepatopancreas and Muscle:

Method:

The glycogen content in the Hepatopancreas and Muscle from each size group individuals of *O. senex senex* subjected to different sublethal (0.2 ppm) fenvalerate exposure periods like 24 hrs, 8-day, 16-day, 24-day and 30-day in relation to the control medium (fresh water without fenvalerate) were estimated by calorimetric method using Anthrone reagent as described by (Carrol *et al.*, 1956). Crabs of the same size were taken and divided into 6 batches of 6 animals each. The entire hepatopancreas from the abdominal region and muscle of the leg of the crab were removed and weighed to the nearest mgs using the microbalance. The hepatopancreas
was then transferred to separate test tubes containing 3.0 ml of hot KOH (Hassid and Abraham, 1957) and digested for 30 minutes. Subsequently, the solution was cooled and to it added 4.0 ml of alcohol. The entire mixture was kept in a refrigerator overnight. The mixture was then centrifuged at 2500 rpm for 15 minutes and the supernatant was completely decanted. 10 ml of warm distill water was added to the residue to dissolve the precipitated glycogen. 0.2 ml of this solution was made upto 10 ml with distill water. The 0.5 ml of Anthrone reagent was added to this solution and the entire mixture was kept in a boiling water bath for 30 minutes. The optical density of the colour developed was measured in spectrophotometer at a wavelength of 625 nm. A blank and glycogen standards were also run similarly. The glycogen content in Hepatopancreas and Muscle in crabs was expressed as mg of glycogen / gm wet weight of the tissue. The mean values of the 6 measurements were taken into account. The maximum % suppression and the % recovery capacity in the hepatopancreatic glycogen were calculated and subjected to statistical analysis.

FENVALERATE EXPOSURE – ENZYMATIC STUDIES

The activity of the enzyme, acetylcholinesterase (AchE) and acetylcholine (Ach) content was estimated separately in the brain, hepatopancreas and muscle from the individuals of O. senex senex at
different sub-lethal exposure periods of fenvalerate. Similar estimations in the tissues of crab in fresh water without fenvalerate served as controls.

Prior to the estimations, the crabs were stunned to death. Then the brain, hepatopancreas and muscle were extracted out from each crab. The tissue samples are weighed individually in an electrical semi microbalance and transferred into separate microbeakers containing crab, ringer solution was prepared as per the composition given by Ekberg (1958). An equilibrium time of 15 minutes is allowed to the tissue samples so as to enable them regain normally from a state of shock, if any, due to handling and dissecting procedures. At the end of this equilibrium period, separate homogenates in the required percentage were prepared from the tissue samples in different enzymes studied. The entire process of preparation of tissue sample was carried out in an ice chamber with a temperature maintenance of $15^\circ c \pm 1^\circ c$ for all the enzymes. Studies in the present investigation, the assays were standardized by preliminarily determining the optimal PH, temperature and substrate concentration using an electrically operated homogeniser and the activity of enzymes were estimated by using the following methods. For this enzymatic studies, the protein content of different tissues was used by the method of Lowry et al., (1951).
METHODS OF ESTIMATION OF ACETYLCHOLINE ESTERASE (ACETYLCHOLINE ACETYLHYDROLASE E.C. 2.1.1.7) AND ACETYL CHOLINE

The activity of acetylcholinesterase (AchE) was separately estimated in the brain, hepatopancreas and muscle from *O. senex senex* at different sublethal exposure periods of Fenvalerate. Similar estimations were carried out in the tissues of *O. senex senex* in fresh water without fenvalerate which served as control. 1% w/v homogenate of the brain and 5% (w/v) homogenate of the other tissues separately prepared in cold 0.25M sucrose solution using an electrically operated homogeniser and the Ach content was estimated by the method Hestrin as given by Augustinson (1957) and Ach content was expressed as μg/gm. wet weight.

FENVALERATE EXPOSURE - ENERGY RICH NUCLEOTIDES:

The concentration of adenylate nucleotides like ATP, ADP and AMP were estimated separately in the brain, hepatopancreas and muscle of the *O. senex senex* at different sub-lethal exposure periods of Fenvalerate. Similar estimations in these tissues of the crab in freshwater without fenvalerate served as control. Prior to the estimations, to stop the movements of the crab once and thereby in order to keep the stationary concentration of the nucleotides unchanged, the crabs were anesthetized with methane sulfonate (MS 222) and transferred in heptan (-90°C) and
thereafter crabs were put immediately in liquid air. Thus the crabs were freezed to death. Then the deep freezed tissues like brain, hepatopancreas and muscle were separated and ground to a fine powder separately in a mortar and a pestle. Separate homogenates were prepared for these tissue samples in ice cold 6% perchloric acid solution. The entire process of isolation and preparation of the tissue sample was carried out in an ice-chamber with temperature maintenance of $15 \pm 1^\circ$C. The homogenate was centrifuged for 10 mts in high speed centrifuge. The extraction procedure results in adenylate recoveries of 95%. From this supernatant, the determination of adenylate nucleotides like ATP, ADP, AMP was carried out according to the method of Bergmeyer (1974). The measurements were carried out in an ELICO spectrophotometer at a wavelength of 360nm and the ATP, ADP, AMP concentrations were expressed in moles/gm wet weight.

**ENERGY CHARGE:**

The adenylate energy charge (AEC) values were calculated by the following formula as given by Atkinson and Walton (1967).

$$\text{AEC} = \frac{\text{ATP} + \frac{3}{2} \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}}$$
Bioaccumulation of Fenvalerate

Now-a-days internal standard method is most popular and the ideal qualitative GLC method of analysis of pesticides. The concentration of the pesticides depend upon the response of Detector to the particular insecticides.

Method

Preparation of Internal Standard Solution (IS)

Weighed accurately 0.25 gm of Di(2-ethylhexyl) Phthalate (DEP) AR Grade and was dissolved in 500 ml of acetone.

Preparation of Standard Fenvalerate Solution

Weighed accurately 0.075gm of standard Fenvalerate technical in 50ml volumetric flask and 5ml of internal standard solution was added. The mixture was shaken and made up to the mark.

Extraction and clean-up process

Tissues of the Fenvalerate treated crab like brain, hepatopancreas and muscle were weighed separately and homogenized with 7ml concentrated formic acid and to this 5ml of n-hexane was added. The contents were shaken and the solvent layer was isolated. Each sample was extracted three times. The solvent layer was washed with glass distilled water and passed through anhydrous sodium sulphate column. The volume
of the extract was reduced to 2ml. 1μl of standard fenvalerate solution and 1μl of extract were analysed by injecting directly into the gas-chromatograph model-8510 equipped with flame ionization detector with the operating conditions of oven temperature 250°C, injection temperature 270°C, detection temperature of 270°C, carrier gas Nitrogen without moisture and oxygen. Column 4mm, SS 3 % ov-101 CHW HP 100-20 mesh. Areas of fenvalerate and internal standard peaks were measured and fenvalerate content was computed and represented in μg/gr/wet weight of the tissue.

Environmental Impact and risk assessment

Environmental impact and risk assessment has recently emerged as a scientific activity for evaluating the toxic nature of a substance and risk is characterised qualitatively as the nature of adverse consequences and quantitatively as their probability (Patric and Anderson 1990). Risk is defined as the nature and probability of occurrence of an adverse effect on an environmental species or media.

Bioconcentration Factor (BCF)

Environmental impact and risk assessment has related to aquatic medium can also be studied by calculating Bio-concentration Factor. BCF is the ratio of the concentration of a chemical in an aquatic organism to the concentration in water at equilibrium. The BCF is an
estimate of the chemical propensity to accumulate in aquatic animals. Chemicals with BCFs 1000 were chosen as having significant potential for Bioconcentration and chemicals with a value < 250 were considered to have a low potential to bioconcentration and chemicals with value of < 100 were considered to be safe (USEPA 1989).

Concentration of the chemical in each part of the crab
BCF = \frac{\text{Concentration of the chemical in water}}{\text{Concentration of the chemical in each part of the crab}}

Hazard Index (HI)

Chronic impacts are quantified as Hazard indices and when the Hazard Index exceeds one, adverse chronic health effects could occur. Hazard Index can be calculated by the formula.

\text{Calculated Daily intake (CDI)}
\text{Hazard Index (HI)} = \frac{\text{Reference Dose / Acceptable Daily Intake (ADI)}}{\text{No observed effect level (NOEL)}}
\text{Acceptable Daily Intake (ADI)} = \frac{\text{CDI}}{\text{HI}}
\text{Uncertainty Factor (UF)}
Recovery studies:

The nature and magnitude of recovery after introduction of a toxicant is, that the toxicant itself must disappear. The extent of recovery can be regarded directly as a good indicator of the rate of adaptation of crab population and an indirect sign of the rate of risk to the environment. Therefore percent recovery in each physiological and biochemical parameter at 30 day sublethal exposure period of fenvalerate was calculated relative to the level of control medium (fresh water without fenvalerate) which was fixed at 100% for the sake of comparison.