# Summary

Herbicides are a powerful weed control tool relied upon by farmers around the world. The use of herbicides to control weeds has been recognized as a part of agricultural practices throughout the world. Unfortunately, the indiscriminate use of these herbicides to improve agricultural production and yield may have impacts on non-target organisms, especially aquatic life forms and their environment. Pyrazonsulfuron ethyl (PE) is one of the herbicide which belongs to sulfonylurea group. This group of herbicides inhibits acetolactate synthase, thereby inhibiting the branch chain Amino Acids and thus inhibiting the growth of weeds. Information pertaining to the metabolism or transformation of this novel herbicide in the environment is poorly documented. The occurrence of sulfonylurea herbicides in aquatic environments is receiving public attention. Residues of sulfonylureas have been detected in surface water and groundwater due to runoff and leaching after their application. Studies till date in our lab has focused on the toxic effects of a variety of agrochemicals including insecticide- Dimethoate, Imidaclorpid; fungicide - cymoxanil and Mancozeb as well as plant nutrient mixture on freshwater fishes. However, approaches to the selective action of pyrazosulfuron-ethyl, a herbicide in physiological and biochemical aspects have been little studied to our knowledge. Hence, the aim of the present study was to evaluate the toxic effect of PE on Freshwater Fish *Oreochromis mossambicus*.

## Chapter I

In controlling the hazardous effects caused due to pesticidal usage, more and more information is needed to characterize its toxicity, than to characterize the risk associated with the use of that chemical in a specific situation. In assessing the safety of pesticides, “animal testing” has become the primary source which yields valid information in monitoring and environmental management. Acute toxicity of a pesticide refers to the effects from a single dose or repeated exposure over a short time. The more the information, the better will be the predication of toxicity in a populations, metabolic state of animals and degenerative changes in the internal parts which are known to influence the toxicity of any compound, therefore toxicity tests have to be carried on healthy and active organisms only. In the present study, 96hr LC50 value was determined. In addition the behavioral response, haematological and biochemical alterations were also explored on freshwater fish *Oreochromis mossambicus* on exposure of PE. The LC50 values according to Probit regression curves was found to be 501.65 mg/l and the Lower Confidence Limit (LCL) value and Upper confidence limit (ULC) were 407.83 mg/l and 595.47mg/l respectively. After 96 h exposure of PE mortality of fishes at 1000 mg/L hundred percent was observed. The exposure of PE resulted into abnormal swimming behaviour (jerky movement, Agitate swimming and lose of equilibrium, increase fin and tail movement). Overall the exposure of PE resulted into hyperactivity and restlessness. The PE exposure resulted into a altered hematological parameters where a significant increase in the RBC count, Haemoglobin and PCV was noted with a decrease in MCHC. Moreover WBC count exhibited a significant increase, and blood glucose as well as protein also illustrated a significant increase in the experimental groups compared to the control group. Through the present study one can conclude that the PE exposure during the acute treatment induces significant changes in the hematological and biochemical parameters of the freshwater fish *O. mossambicus*. The alterations of the parameters may provide the early sign for the determination of acute toxic level of herbicide and their effects on aquatic medium. The findings of present study also provide a better understanding of toxicological endpoint of aquatic pollutants and safer level of these herbicides in the aquatic environment and protection of aquatic habitats.

**Chapter II**

On basis of $LC_{50}$ value sub-acute study dose $1/20^{th}LC_{50}$ (Low dose), $1/10^{th}$ $LC_{50}$ (Medium dose), $1/5^{th}$ $LC_{50}$ (High dose) were chosen. Haematological (RBC, HB, PCV, MCV, MCHC, MCH, Total WBC count) and biochemical (Protein, Albumin, Globulin, Glucose, BUN, Urea and Creatinine ) examinations of the experimental as well as the control fish were conducted at $7^{th}$ and $14^{th}$ days of exposure. From all the groups after 14 days of exposure the remaining fish were shifted to herbicide free fresh water and blood samples were analysed at 28 days of recovery. Fish exposed to sub-lethal levels of PE resulted in a significant time- and dose-dependent decrease in total erythrocyte count, haemoglobin content, and hematocrit values, and increases in total leukocyte count. During a subsequent recovery study period of 28 days, the haematological parameters as well as the biochemical titres were found to get normal values which can be interpreted as either a compensatory response by the fish against the toxic conditions, or insufficient recovery time to elicit a complete recovery. The improvement in haematological profile and biochemical parameters of the test fish when transferred to herbicide-free freshwater suggests that PE entering into the system is slowly eliminated and hence the haematological and biochemical parameters recover from the herbicide toxicity. The results suggest that 28 days are insufficient for complete recovery to PE exposure by *O. mossambicus* and a longer period would be required for full recovery. Moreover, the study showed that the recovery phase following PE exposure could change biochemical parameters to levels that are not close to those seen during exposure or control levels.

**Chapter III**

The condition factor (K) of fish is a parameter which is used widely in order to understand survival, reproduction, maturity and health of fish, and often is a good indicator of water quality or general health of fish populations. Organosomatic indices are described as the ratios of organs to body weight, and measured organ in relation to body mass which can be directly linked to toxic effects of chemical on target organ. An attempt is made to study the alterations in the organosomatic index (HSI, GSI, KSI, SSI and CSI) as well as the condition factor (K) of freshwater teleost fish, *Oreochromis mossambicus* on exposure of the herbicide PE. The CF in the present study reflected a significant decrease in dose and time dependent manner. A decrease in CF can be due to an indirect effect of toxicant on macromolecular syntheses which are secondary effects induced by physiological stress. The sub lethal concentrations of PE used in this study produced significant changes in the condition factor, hepatosomatic, cardiosomatic, renatosmatic, splenosomatic indices in *O.mossambicus*. As a general indicator of the overall health and well-being of the fish, alterations in the organosomatic indices and condition factor indicate deleterious effect of the herbicide PE. Overall, PE exposure had resulted into an altered health status of *O.mossambicus*. Thus, Herbicide PE should be used with caution in the environment since its careless use may lead to acute exposure to terrestrial and aquatic organisms thereby destabilizing the natural ecosystem. Further, CF and organosomatic indices can be considered as an integrative bioindicators for investigating the stress of the toxicant. Our study is significant because this is the first report for the alterations in the organosomatic indices and CF of *O.Mossambicus* for PE. Additionally, the concentration that caused these changes was much lower than anticipated. This shows that anywhere that herbicide runoff is present; it is possible that the organism in that microenvironment will suffer negative effects from exposure to even low levels PE. The use of this toxicant in the environment should be done with caution.

**Chapter IV**

Qualitative and quantitative assessment was carried out to describe the toxic induces histological changes in gills, liver and kidney organs of *O.Mossambicus* after sub-acute exposure of PE. Time and dose dependent alteration in the reaction pattern was obtained. Histological alterations were confirmed by the semiquantitative analysis, which includes circulatory disturbances, regressive changes and progressive alterations. On the basis of the reaction pattern, the organ index of all the tissues after 7 days of PE exposure exhibited a slight histological alteration which represents class I. However after $14^{th}$ day, at low dose; Gills, liver and Kidney exhibited moderate histological alterations which represent class II. At medium dose, all the tissues exhibited severe histological alterations which represent class III, and at High dose, Gills, Liver and Kidney exhibited pronounced histological alterations which represent class IV. Class I and II stages are classified as repairable, signifying that the LD and MD has resulted into histological alterations that are reversible changes. However, an increased frequency of histological alterations is observed at LD, MD and HD after 14 days, indicating dysfunctions induced by PE, since metabolically active areas of the liver as well as gills are reduced, leading to a possible reduction in the overall functions performed by this organs. Overall, the histopathological lesions in all three tissues recorded at two different time period and three doses demonstrate the cumulative physiological and biochemical effects of PE exposure. The effects were more pronounced after 14 days and exhibited regressive changes in gills, liver and kidney compared to 7 days. Of all the organs, it was kidney that revealed severe alterations compared to liver and gills at HD in comparison to the MD and LD. In conclusion, the present study indicates that sub acute exposure to PE induces histopathological alterations in gills, liver, and kidneys. Overall, the study demonstrated that at sub-lethal concentrations of PE used after $14^{th}$ day, the amount of cellular change in the Gills, liver and kidney tissue of *O.mossambicus*, did affect in a severe and irreversible way for this hardy fish. The combination of semiqunatitative histological study proved to be a useful tool for investigating sublethal effects of environmental contaminants.

**Chapter V**

An attempt was made to verify genotoxic potential of herbicide PE in peripheral blood erythrocytes of *O.mossambicus*. To prove the clastogenic and aneugenic potential micronucleus was performed along with the alteration in the frequency of micronucleus and other morphological abnormalities in erythrocytes. Apoptotic characteristics can be quantitatively examined by flow cytometry, the preferred platform for rapid assessment of multiple cellular attributes at a single cell level. The major advantages of flow cytometry include the possibility of multiparameter measurements; single cell analysis, and rapid analysis time Hence, flow cytometry was also performed. A significant time and dose dependent increase in the frequency of MN in peripheral blood erythrocytes of fish treated with herbicide PE was detected compared with control group of fish. At 7 day exposure, there was linearity in the increase in the apoptotic population of erythrocytes i.e. from LD to HD. While, this linearity was also observed for 14 days exposure period. The highest cell death was accounted for HD of both the durations suggesting cells were under stressed which lead them to undergo apoptosis at sub lethal exposure of PE. The MN studies and FACS results thus postulates that erythrocytes have entered the apoptosis; together these data show that PE exposure is potentially hazardous to *O.mossambicus*, even at sub-lethal levels as evidenced by the cell death study which was found to be significantly increasing in a dose and time dependent manner. Thus, it can be summarized that it is always advisable to simultaneously study more than one marker to provide a multidimensional view of advancing apoptotic cascade. Further, the results of MN and FACS also suggest that in field, even the sub-lethal concentration can cause toxicity to fresh water fish and results in altering the aquatic ecosystem.