Fresh Water Fish As Biomarkers/Bioindicators For Pesticide Pollution

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Introduction

Increasing awareness of the toxicological potential of pesticides in the environment has created a need for reliable monitoring of these substances in air, soil and especially water. Conventional analytical techniques, although highly precise, are costly and require trained personnel. Biosensors on the other hand, are specific, give fast response times, of low cost and easily used because it is portable. This can present distinct advantages in certain cases (Sanchez, et al, 1997).

Previous experiments in which fresh water fish was exposed to chronic levels of pesticides caused changes in key nitrogen metabolizing enzymes as well as acetylcholinesterase (Abe Zeid et al, 1998). This indicated that other enzymes might also be similarly affected, in particular the enzymes used as biomarkers in other species. The comparative effects of sublethal concentrations of a selection of pesticides on the hepatic enzymes, e.g. glutathione transferase (GST), key esterases, acetylcholinesterae and other enzymes will be studied. Exposure to low concentrations of the pesticides will induce or inhibit the biotransformation enzymes. The responses will be evaluated as biomarkers for contamination of the pesticides in the aquatic environment. Information from these studies can be used to determine the water quality criteria essential for commercial and culture of fresh water fish. The data obtained can also be used to assess or determine the biomarkers which are most suitable to assess inland water pollution by pesticides and to classify the environmental quality of the sites

Materials and Methods

Estimation of LC_{50} value of carbofuran on the African catfish, *Clarius gariepinus*

The African catfish. *Clarius* gariepinus was exposed to various concentrations of carbofuran to determine the toxicity of the pesticide to the fish. The LC_{50} value for endosulfan was already determined in earlier project.

2. Toxic effects of endosulfan and carbofuran on enzyme activities in different organs of catfish.

Separate experiments in which fish was exposed sublethal concentrations of carbofuran and endosulfan (0.82ppm and 10.2 ppm respectively) for 4, 8, 12, 16 and 20 days were carried out. Enzymes Glutathione-S-transferase (GST), Glutathione Peroxidase (GPx), and 7-Ethoxyresorufin O-Dieethylase (EROD) from the liver and different parts of the intestine of control and experimental fish at the specified time intervals were extracted and assayed.

Effects of pesticides on the bacteria *R,melliloti*

The bacteria were exposed to various concentrations of the pesticides. Very inconsistent results on the concentration of pesticides that was sensitive to the bacteria. Experiments were terminated because of the time constraint. However, this will be continued in ongoing projects.

Results and Discussion

The LC_{50} of carbofuran for the African catfish, *Clarius gariepinus*, was estimated to be 1.63ppm. The lethal concentration for 100% mortality was 2.25ppm while the safe concentration where there was no mortality was 1.25ppm. The corresponding figures for endosulfan for the African catfish which were determined in the earlier project were 21.47ppb, 30 ppb and 16ppb respectively

Effects of Carbofuran

There was a general decrease in GST activity in the liver and the small intestine of fish treated with carbofuran after exposure for 21 days. The declining trend after prolonged exposure period could possibly due to the carbofuran being hydrolyzed to 3-hydroxycarbofuran. Previous studies have shown a high percentage of carbamates were eliminated in the excreta of hens as hydrolysis products . GPx activity increased during the first 16 days after which it decreased. The decrease in GPx activity may be due to lipid peroxidation via the consumption of these enzymes for prevention of peroxidation stress . EROD activity on the other hand increases until day 4 and then decreased until the end of the experimental period. These results indicated that the African catfish can metabolize carbofuran initially but subsequently the system is overwhelmed by the pesticide by the direct action of the pesticide on the synthesis of EROD, resulting in the decrease of the enzyme activity on prolonged exposure. Results from the study also show that EROD activities in the liver and proximal intestine are more sensitive to carbofuran than the medial and distal intestines. These correlated well with previous studies on induction of EROD by carbofuran in cultured catfish hepatocytes.

Effects of Endosulfan

GST, GPx and EROD activities were induced in the presence of sublethal concentrations of endosulfan. This is consistent with previous studies on other fishes. It is not surprising since organochlorine compounds are common inducers of cytchrome

P450. Endosulfan had been shown to induce hepatic GST activity in mice and two Lepidoptera and insects . Information on the potential effects on xenobiotics metabolizing enzymes of subtoxic doses of endosulfan in freshwater fish showed that it may be a mixed-type inducer because of the significant increase of EROD, GST anf GPx activities.

Comparison of Enzyme Activity in Fish Exposed to Endosulfan versus Carbofuran

Generally, the enzyme activity in fish exposed to endosulfan was higher than those exposed to carbofuran during the entire experimental period and in all fish organs. This is probably because endosulfan has lower solubility (0.32mg/l) compared to carbofuran (320mg/l). This condition enables the carbofuran to be readily excreted from the body of treated fish compared to endosulfan

These results on the induction of GST, GPx and EROD activities when exposed to endosulfan and carbofuran demonstrated that fish intestine and liver were sensitive to environmental exposure

Conclusions

The present results indicate that induction of the various enzyme activities by carbofuran and endosulfan in fish tissue may be suggested as biomarkers of aquatic pollution. EROD activity in particular is very sensitive to the presence of pesticides as compared to GST and GPx. The present results also indicate that the small intestine may be more sensitive compared to the liver. The distal segment of the intestine strongly suggests a site for potential biomarkers for endosulfan and carbofuran

Benefits from the study

The information is important for the diagnosis of the status of the environment.

Consumers should be made aware of the potential hazards of these pesticides through the consumption of fish where pesticides are indiscriminately used.

The possibility of using fresh water fish as biological indicators of hazardous chemicals. e.g. *Products/processes/technologies*

Patent(s), if applicable-:

Nil

Stage of Commercialization, if applicable-:

Nil

Project Publications in Refereed Journals

1. Abu Zeid IM, Shamaan NA, Arshad JH, Ramli J, Omar I and **Syed MA.** 1999. Effect of endosulfan on the liver of African catfish, Clarius gariepinus: A biological marker approach. Malaysian Journal of Biochemistry and Molecular Biology.4:52.

2. Daryani, Shamaan NA, Arshad JH, Ramli J and **Syed MA.** 2001. Effect of Endosulfan on the African Catfish. 2001. Malaysian Journal of Biochemistry and Molecular Biology. 6: 80

3. Shamaan NA, Abu Zeid IM, Ramli J, Arshad JH, Omar I and **Syed MA.** 2002. Carbofuran and endosulfan toxicities, amino acid transaminase and acetycholinesterase activities in the African catfish, Clarius gariepinus (accepted Pertanika J Sci tropical Agric 2002

Project Publications in Conference Proceedings

1. Daryani, **Syed, MA,** Shamaan Na, Arshad JH and Ramli J. 2002. The effect of endosulfan and carbofuran on the African catfish *Clarius gariepinus*. Abstracts of the 27th Annual Conference of the Malaysian Society for Biochemistry and Molecular Biology, Pan Pacific Hotel, Kuala Lumpur, 7th October 2002; p 76

2. Daryani, **Syed MA,** Shamaan NA, Arshad JH and Ramli J. 2001. Effect of endosulfan on the African catfish *Clarias gariepinus*. Abstracts of 26th Annual Conference of the Malaysian Society for Biochemistry and Molecular Biology, Crown Princess Hotel, Kuala Lumpur, 2nd-3rd October 2001

3. Abu Zeid IM, Shamaan NA, JH Arshad, J Ramli, I Omar and **MA Syed.** 2000. Biochemical changes in the liver of African catfish *Glarias gariepinus* as indicators of pesticide stress. Abstract of 25th Annual Conference of the Malaysian Society for Biochemistry and Molecular Biology, Sheraton Imperial Hotel, Kuala Lumpur, 3rd October 2000 p 20.

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year

Isam El Din Abu Zeid	**The Effect of Carbofuran and Endosulfan on teh African catfish, *Clarius gariepinus***	**Pesticide Biochemistry**	**PhD**	**2001**
Daryani	**Effect of Endosulfan And carbofuran on enzyme activities in *Clarius gariepinus* (African catfish)**	**Pesticide Biochemistry**	**Masters of Science**	**2003**

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