Journal of Chemical and Pharmaceutical Research



J. Chem. Pharm. Res., 2011, 3(3):397-402

Bioaccumulation Study on the Cypermethrin Toxicity in Fresh Water Field Crab, *Spiralothelphusa hydrodroma*(Herbst)

R. S. Sreenivasan^{1*}, P. Krishna Moorthy² and M. Deecaraman³

¹Department of Chemistry, VELTECH MULTITECH Dr. Rangarajan Dr. Sakunthala Engineering College, Chennai, India ²Department of Bioinformatics, Bharath University, Chennai, India ³Department of Industrial Biotechnology, Dr. MGR University, Chennai, India

ABSTRACT

The fresh water field crab, Spiralothelphusa hydrodroma is an important human food source in parts of South India and the crab is constantly exposed to pesticides, which are used extensively to control agricultural pests. Evaluation of the toxic effect of cypermethrin on the experimental crab for the LC₅₀ value was carried out. Effect of cypermethrin on the quantitative study of the nutritive value viz., protein, carbohydrate and lipid in ovary, spermatheca, hepatopancreas, muscle, gills, haemolymph, brain, thoracic ganglia and eyestalk was studied. It is concluded that the accumulation of cypermethrin in the hepatopancreas is found to be exponential in an environment of cypermethrin which is commonly used as biocides and fertilizers.

Keywords: Cypermethrin, LC_{50} , *Spiralothelphusa hydrodroma*, ovary, spermatheca, hepatopancreas, muscle, gills, haemolymph, brain, thoracic ganglia and eyestalk.

INTRODUCTION

The pesticides include insecticides, herbicides, fungicides, molluscides, nematicides and heavy metals like copper, zinc, arsenic, lead, cadmium, mercury etc[1]. These pesticides are nonbiodegradable and accumulate in the food chain. Mostly they are prone to affect the nervous system causing tumors in living organisms. They are not only neurotoxic but also affect other systems and have shown a high degree of impact on metabolism by altering the protein, carbohydrate and lipid[2, 3]. The trace metal concentration in Queensland Estuarine crabs, Australoplax tridentate and Scylla serrata has been observed[4]. The present work aims to study the effect of bioaccumulation of cypermethrin in different tissues of Spiralothelphusa hydrodroma(Herbst).

EXPERIMENTAL SECTION

The fresh water field crabs were collected from, in and around the irrigating channels and paddy fields. The crabs were maintained in normal daylight illumination in the laboratory thereby providing normal acclimatization. The crabs were fed with uncooked oats. For all experiments, the crabs were used with carapace length ranging from 3 to 4.5cm and breadth ranging from 5 to 6.5cm. The water level was maintained carefully so that the crabs were partially immersed. Acute toxicity study was carried out to determine the potency of cypermethrin for static but renewal type of bioassay was adopted in the present investigation to estimate the LC₅₀ values. The cypermethrin, commercial grade was used as the test material since only commercial preparation is used in agriculture. The experiment was carried out to find the range of concentrations for confirmatory evaluation. The mortality was recorded for the crab at 24, 48, 72 and 96hr exposure to cypermethrin were corrected for natural response by Abbott's formula[5]. The LC₅₀ values for 24, 48, 72 and 96hr of exposure periods were estimated as 2.027, 1.698, 1.452 and 1.315ppm respectively.

Exposure	LC ₅₀	Upper	Lower	Regression results	Slope	r^2
period	(ppm)	confidence	confidence		function	
(hours)		limits (ppm)	limits (ppm)			
24	2.027	2.561	1.739	Y = -0.932 X + 0.468	2.973	0.99
48	1.698	1.938	1.345	Y = -0.658 X + 0.281	3.265	0.98
72	1.452	1.883	1.136	Y = -0.724 X + 0.391	4.121	0.99
96	1.315	1.763	1.118	Y = -0.611 X + 0.324	4.973	0.99

Table 1 The LC_{50} values and regression equations for S. hydrodroma treated with cypermethrin

Chronic time course study on the effect of cypermethrin on the crab was conducted by exposing to two sublethal, safe concentrations for 20 and 40 days. According to Matsumura[6] and Rao and Rao[7], $1/10^{th}$ and $1/3^{rd}$ of the 96hr LC₅₀ value represent lower and higher sublethal concentrations respectively. Hence lower (0.1315ppm) and higher (0.4383ppm) sublethal concentrations of the insecticide were arbitrarily used. At the end of the treatment period, the cypermethrin treated crabs were dissected and the tissues namely ovary, spermatheca, hepatopancreas, muscle, gills, haemolymph, brain, thoracic ganglia and eyestalk were weighed (250mg) and digested in concentrated nitric acid and perchloric acid in the ratio of 3:1. The mixture was evaporated to near dryness and then resuspended in 5ml of 50% HNO₃ and was filtered in a millipore unit and made upto 25ml with metal free double distilled water. The digested samples were aspirated into an atomic absorption spectrophotometer[8]. One-way Analysis of Variance (ANOVA) was performed based on the methods of Winer[9].

RESULTS

The results have fascinating informations on the bioaccumulation of cypermethrin at lower (0.1315ppm) and at higher (0.4383ppm) subletal concentrations on the ovary, spermatheca, hepatopancreas, muscle, gills, haemolymph, brain, thoracic ganglia and eyestalk.

Exposure		Lower sublethal	Concentra	Higher sublethal	Concentra
period in		concentration	tion factor	concentration	tion factor
days	Tissues	Mean \pm SD	(C.F)	Mean \pm SD	(C.F)
20	Ovary	1.14 ± 0.17	0.0087	1.66 ± 0.22	0.0039
	Spermatheca	0.71 ± 0.18	0.0054	1.23 ± 0.31	0.0028
	Hepatopancreas	2.43 ± 0.09	0.0187	3.22 ± 0.17	0.0075
	Muscle	0.92 ± 0.13	0.0070	1.98 ± 0.13	0.0046
	Gills	1.26 ± 0.12	0.0096	1.78 ± 0.09	0.0041
	Haemolymph	1.58 ± 0.13	0.0121	2.33 ± 0.08	0.0054
	Brain	0.37 ± 0.08	0.0028	0.86 ± 0.07	0.0020
	Thoracic ganglia	0.52 ± 0.06	0.0040	0.98 ± 0.10	0.0023
	Eyestalk	0.25 ± 0.04	0.0019	0.48 ± 0.03	0.0011
40	Ovary	1.39 ± 0.18	0.0107	2.03 ± 0.19	0.0047
	Spermatheca	0.93 ± 0.12	0.0072	1.89 ± 0.10	0.0044
	Hepatopancreas	2.82 ± 0.13	0.0217	3.94 ± 0.14	0.0092
	Muscle	1.24 ± 0.23	0.0095	2.23 ± 0.13	0.0052
	Gills	1.48 ± 0.09	0.0114	2.46 ± 0.13	0.0057
	Haemolymph	1.81 ± 0.11	0.0139	2.79 ± 0.08	0.0065
	Brain	0.62 ± 0.07	0.0048	0.96 ± 0.12	0.0022
	Thoracic ganglia	0.84 ± 0.18	0.0065	1.28 ± 0.07	0.0029
	Eyestalk	0.39 ± 0.06	0.0030	0.84 ± 0.06	0.0019

Table 2 Bioaccumulation of cypermethrin among various tissues of S.hydrodroma exposed to lower
(0.1315ppm) and higher (0.4383ppm) sublethal concentrations

Mean ± SD of six individual observations Values are expressed µg/g wet tissue and µg/ml haemolymph In control, cypermethrin level not traced out C.F. – tissue concentration of cypermethrin/concentration of cypermethrin in test water

Ovary

The sublethal concentrations of cypermethrin was analysed for the bioaccumulation in the ovary of treated crabs. In the crabs treated with lower sublethal concentration (0.1315ppm) of cypermethrin the bioaccumulation was 1.14 and $1.39\mu g/g$ fresh weight. However, when the cypermethrin concentration increased to higher sublethal level (0.4383ppm) the bioaccumulation drastically increased to 1.66 and 2.03 $\mu g/g$ wet weight of tissue for 20 and 40days respectively.

Spermatheca

When the crab exposed to lower sublethal concentration (0.1315ppm) of cypermethrin, the bioaccumulation in spermatheca was 0.71 and $0.93\mu g/g$ fresh weight of tissue whereas in higher sublethal concentration (0.4383ppm) the bioaccumulation was 1.23 and 1.89 $\mu g/g$ wet weight of tissue for 20 and 40days respectively.

Hepatopancreas

As observed from the results, the bioaccumulation of cypermethrin in the hepatopancreas of lower sublethal concentration (0.1315ppm) treated crabs was 2.43 and $2.82\mu g/g$ wet weight of tissue, whereas in higher sublethal concentration (0.4383ppm) the bioaccumulation was 3.22 and $3.94\mu g/g$ wet weight of tissue.

Muscle

As from the results, the bioaccumulation of cypermethrin in the lower sublethal concentration (0.1315ppm) of cypermethrin treated crabs was 0.92 and $1.24\mu g/g$ fresh weight of tissue,

R. S. Sreenivasan et al

whereas higher sublethal concentration (0.4383ppm) the bioaccumulation was 1.98 and $2.23\mu g/g$ fresh weight of tissue for 20 and 40days respectively.

Gills

When the crab was exposed to lower sublethal concentration (0.1315ppm) of cypermethrin, the bioaccumulation in gills was 1.26 and $1.48\mu g/g$ fresh weight of tissue, whereas in higher sublethal concentration (0.4383ppm) the bioaccumulation was 1.78 and $2.46\mu g/g$ wet weight of tissue for 20 and 40days respectively.

Haemolymph

The bioaccumulation of cypermethrin in the haemolymph increased drastically as the concentration of cypermethrin increased. In the lower sublethal (0.1315ppm) concentration the bioaccumulation of cypermethrin was found to be 1.58 and 1.81μ g/ml, whereas in higher sublethal concentration (0.4383ppm) the bioaccumulation was 2.33 and 2.79 μ g/ml for 20 and 40days respectively.

Brain

As observed from the results, the bioaccumulation of cypermethrin in the brain of lower sublethal concentration (0.1315ppm) treated crabs was 0.37 and 0.62 μ g/g wet weight of tissue, whereas in higher sublethal concentration (0.4383ppm) the bioaccumulation was 0.86 and 0.96 μ g/g wet weight of tissue.

Thoracic ganglia

When the crab was exposed to lower sublethal concentration (0.1315ppm) of cypermethrin, the bioaccumulation in thoracic ganglia was 0.52 and $0.84\mu g/g$ fresh weight of tissue, whereas in higher sublethal concentration (0.4383ppm) the bioaccumulation was 0.98 and $1.28\mu g/g$ wet weight of tissue for 20 and 40days respectively.

Eyestalk

When the crab was exposed to lower sublethal concentration (0.1315ppm) of cypermethrin, the bioaccumulation in eyestalk was 0.25 and $0.39\mu g/g$ fresh weight of tissue, whereas in higher sublethal concentration (0.4383ppm) the bioaccumulation was 0.48 and $0.84\mu g/g$ wet weight of tissue for 20 and 40days respectively.

DISCUSSION

In general, fall in carbohydrate level may be due to prolonged exposure of the metabolism to heavy metals and this may be the reason for inactivation of the enzyme, involved in the carbohydrate metabolism. This has been reported[10] in the hepatopancreas and muscle. Depletion of haemolympth glucose, tissue glycogen and total free sugars has been reported[8] in the case of long exposure to chromium contamination. Decline in glycogen content has been reported[11] in the case of long exposure to zinc contamination. It has also been reported[12] to have significant changes in the catabolism of carbohydrate in the tissues on prolonged exposure to methyl parathion. If the exposure is towards cadmium the carbohydrate is found to be decreased and this is due to *Scylla serrata*[13]. If the exposure is made to cadmium and mercury the carbohydrate is found to be decreased[14]. Fall in carbohydrate levels after prolonged

exposure to heavy metals polluted water was due to the inactivation of the enzyme involved in the carbohydrate metabolism[15]. The activity of the enzyme phosphorylase in the hepatopancreas and muscle has been shown to reduce the carbohydrate levels in *O.senex*[16].

The accumulation of cypermethrin in the present study, in the tissues was in the order of hepatopancreas, haemolymph, gill, ovary, muscle. Maximum accumulation of cypermethrin in the hepatopancreas in various crustaceans was reported by many workers namely in *Penaeus duorarum* in response to cadmium[17]; *Rangia cuneata* exposed to mercury[18]; *Potamonautes warreni* exposed to zinc and lead[19]; *Macrobrachium malcolmsonii* in response to nickel[20].

The lipid content level is also found to be decreased in hepatopancreas. This is due to accelerated hydrolysis of lipid and this happens because of high energy demand that occurs due to metal toxicity. Among the biomolecules carbohydrate, protein and lipid are the main ones that are in action at times of stress due to toxicity of chemicals.

CONCLUSION

It is concluded that the accumulation of cypermethrin in the hepatopancreas is found to be exponential in an environment of cypermethrin which is commonly used as biocides and fertilizers. The accumulation takes place at both sublethal levels viz, lower (0.1315ppm), higher (0.4383ppm). Fall in carbohydrate is due to prolonged exposure of the metabolism to pesticides and during this process the enzymes involved in the carbohydrate metabolism are inactivated. The lipid level is also found to be decreased in hepatopancreas and this is due to accelerated hydrolysis and this happens because of the high energy demands that occur due to metal toxicity.

Acknowledgement

Authors sincerely thank Dr.R.Rangarajan, Chairman, Dr.Sakunthala Rangarajan, Vice-Chairman, VELTECH Group of Educational Institutions and Dr.K.Siddappa Naidu, Principal, VELTECH MULTITECH Dr.Rangarajan Dr.Sakunthala Engineering College, Chennai–600062 for their unremitting encouragement for publishing this paper successfully.

REFERENCES

[1] S Jayakumar. Effects of Copper and Zinc Toxicity on a Fresh Water Crab, *Spiralothelphusa hydrodroma*. Ph.D. Thesis, University of Madras, India, **2002**.

[2] MJ McKee; CO Knowles, *Ecotoxicol. Environ. Saf.*, 1986, 12, 70-84.

[3] R Nagabhushanam; J Deshpande; R Sarojini, Proc. Natl. Symp. Ecotoxicol., 1972, 73-84.

[4] MR Mortimer, Marine Pollution Bulletin, 2000, 41(7-12), 359-366.

[5] WS Abbott, J. Econ. Entomo., 1925, 18, 265-267.

[6] F Matsumura. Toxicology of Insecticides. Plenom Press, New York, 1975.

[7] JR Rao; KVR Rao, Proc. Indian Natl. Sci. Acad., 1981, 47, 53-58.

[8] F Van Hoof; M Van San, *Chemosphere*, **1981**, 10(10), 1127-1135.

[9] BJ Winer. Statistical Principles in Experimental Design II. McGraw-Hill, New York, 1971.

[10] K Vijayaraman; G John; P Sivakumar; RR Mohammed, J. Environ. Biol., 1999, 20(3), 217-222.

[11] R Sarojini; PR Machale; R Nagabhushanam, J. Natl. Conserv., 1990, 2, 97-104.

[12] PS Reddy; KVR Rao, Bull. Environ. Contam. Toxicol., 1991, 47, 925-932.

[13] PS Reddy; A Bhagyalakshmi, Ecotoxocol. Environ. Saf., 1994, 29(3), 255-264.

[14] V Suresh. A Study on the Effects of Heavy Metal Toxicity on a Brackish Water Crab, *Uca annulipes*(Celuca) of Pulicat Lake, Tamil Nadu. Ph.D. Thesis, University of Madras, India, **2001.**

[15] R Nagabhuhanam; GK Kulkarni, Aquaculture, 1981, 23, 19-27.

[16] R Ramamurthi; D Venkataramaniah, Comp. Physiol. Ecol., 1982, 7(2), 65-67.

[17] DWR Nimmo, DV Lightner, LH Bhaner. Physiological Response of Marine Biota to Pollutants. Academic Press, New York, **1977**, 131-183.

[18] TM Dillon; JM Neff, Mar. Environ. Res., 1978, 1, 67-77.

[19] HM du Preeze; VE Steenkamp; HJ Schoonbee, *Proc. Euro. Conf. Ecotoxicol.*, **1993**, 1&2, 469-478.

[20] P Kabila; P Saravanabhavan; P Geraldine, J. Environ. Biol., 1999, 20(4), 307-312.