April 2008 *Volume 3 Issue 2*

Trade Science Inc.

Environmental Science

An Indian Journal

Current Research Paper

ESAIJ, 3(2), 2008 [177-180]

Effects of fipronil on freshwater shrimp (*caridina japonica*): Acute toxicity and acetylcholinesterase activity

Jen-Lee Yang[1, 2]*, Liang-Hsien Chen[1], Ying-Chou Lee[3], Hon-Cheng Chen[3]

[1]Department of Life Science, Chinese Culture University, Taipei, 111, R.O.C, (TAIWAN)

[2]Environment Education Counseling Division, Taipei County Government, Taipei, 220, R.O.C, (TAIWAN)

[3]Institute of Fisheries Science, National Taiwan University, Taipei 106, R.O.C, (TAIWAN)

Tel: 886-2-2955-1291; Fax: 886-2-2954-0962

E-mail : d88225003@mail.anhoes.tpc.edu.tw

Received: 30th January, 2007 ; Accepted: 5th February, 2007

ABSTRACT

The purposes of this study were to investigate the effects of fipronil on acute toxicity as well as on acetylcholinesterase(AChE) activity in the hemolymph of freshwater shrimp(*Caridina japonica*). Median lethal concentrations were determined in acute toxicity tests. The 96-h LC_{50} value was 0.086(0.061-0.121)g/L. During 3 weeks testing period, *C.japonica* were exposed to 3 different sublethal levels of fipronil(0.01, 0.02, and 0.04μg/L) in laboratory toxicity tests. A decrease of AChE activity in hemolymph was observed, particularly at the highest exposure concentrations.

KEYWORDS

Fipronil;
Acute toxicity;
Acetylcholinesterase;
Caridina japonica.

INTRODUCTION

Fipronil($C_{12}H_4Cl_2F_6N_4OS$)is a member of a phenylpyrazoles, as one of the first phenylpyrazoles introduced for pest of corn, rice, and turf, and for control of cockroaches, fire ants, termites, and fleas. Fipronil is one of the most persistent, lipophilic, insoluble in water and is readily phototransformed by sunlight to a variety of metabolites one of which fipronil-desulfinyl is extremely stable and is more toxic than the parent compound[4]. Two major degradation products of fipronil are fipronil sulfone and fipronil-desulfinyl, each possessing high insecticidal activity[3]. Fipronil is also a neurotoxic chemical, chief inhibitory neurotransmitter in the central nervous system, it is known to block gamma-aminobutyric acid(GABA) receptors and interferes with the passage of chloride ions through a GABA-gated channel. At low concentrations, it disrupts nervous system function of organism. At higher doses lead to neural excitation and eventually death of the biota causes death[5,14].

All chemicals are harmful if they are present in high enough concentration, even those chemicals that are essential to life at lower concentrations. Determinations of chemical lethal concentration(LC_{50}) could provide objective information to establish water quality criteria of certain toxic substances[10]. Observations of changes in locomotor activity in response to the presence of a pollutant tend to be important and often are associated with other studies. In a real sense, nearly all behaviors

of aquatic animal involve locomotor activity. Indeed, it is the predictable body movements of an animal that the behavioral scientist observes and uses to draw conclusions as to purpose, causality, etc. Kleerekoper[9] indicated has shown these movement patterns to be a nonrandom process under central nervous system control. The treated animals are described as becoming restless, excitable, lethargic, and dashing wildly. These subjective observations can be of some qualitative use, but it is much better when the extent of bodily activity can be quantified. However, various methods offer a way of quantifying treated animal locomotor activity while measuring some other physiological indices such as oxygen consumption, heart rate, and neurotransmitter measuring[1].

Like other organophosphate and carbamate insecticides, phenylpyrazole compounds work by interfering with the activity of acetylcholinesterase(AChE), the enzyme responsible for hydrolysis of the neurotransmitter acetylcholine(ChE) at the neuromuscular junction. Inhibition of this enzyme leads to accumulation of the neurotransmitter ChE attached to its receptor, thus over-stimulating the nerve and leading to prolonged muscle contraction, eventually resulting in mortality. AChE is widely distributed among vertebrate and invertebrate animals and inhibition of ChE activity is a specific biological indicator of exposure to agricultural pesticides with fewer reports on aquatic inverbrates[15].

Estuaries are among the world's most productive ecosystems, but they are also important as nursery grounds for many ecologically and economically important species[6]. Fipronil has a high affinity for sediment carbon and organismal lipid and a half-life of >5 years[4]. Due to its high hydrophobicity and long half life places it among those toxicants of highest ecological concern in muddy estuarine settings where it is most likely to accumulate. However, there is limited toxicity data exists for fipronil on non-target organisms, especially invertebrate those who were inhabited aquatic systems. Freshwater shrimp (*Caridina japonica*), is a common aquatic invertebrate widely distributed in downstream of rivers throughout eastern Asia-Pacific area and constructs a primary connection in the freshwater ecological chain. The purpose of this study was to examine the effects of fipronil on acute toxicity, AChE enzyme activities in hemolymph of the *M. nipponense*, and can be an indicator to detect the pollution of neuroendocrine-discruption factors in aquatic organisms.

EXPERIMENTAL

Freshwater shrimp(*Caridina japonica*) were obtained from the local commercial suppliers. *C.japonica* were transported to the glass aquarium in our laboratory which was equipped with a water-cycling device; dechlorinated tap water(pH 7.4-8.1; dissolved oxygen concentration 7.3-7.8mg/L; hardness 38-45 $CaCO_3$ mg/L) was used during the entire experiment. The temperature was maintained at 23-25^{0}C, and the photoperiod was set at 12h of light and 12h of dark. They were acclimated for 2 weeks and fed aquarium shrimp mixture everyday. Shrimp(1.2±0.18cm in fork length) were used in the initial experiments. Fipronil was purchased from Sigma (St.Louis, MO). Stock solutions were prepared in acetone and stored under 4^{0}C for use.

Laboratory static renewal tests were conducted to determine the median lethal concentration(LC_{50}) for *C.japonica*. Ten animal of similar size were randomly sampled and placed in 10-L glass beakers. After 24h of acclimatization, shrimp were exposed to different fipronil(0,0.005, 0.01, 0.05, 0.1, 0.2, 0.4, and 0.6mg/L for 96h or more, respectively. The control and each treated group were run in duplicate. During the experiment, dead animal were removed, and mortality was recorded after 24, 48, 72, and 96 h. The LC50 of every test chemicals and their 95% confidence limits for shrimp were calculated using a Basic program from the probit analysis described by Finney[7].

C.japonica for sublethal tests were randomly placed in 20-L glass aquaria. Every aquarium contained 20 shrimp which were exposed to the following concentrations: 0.0, 0.01, 0.02, and 0.04μg/L, respectively in triplicate. Twice a week 50% of the water was renewed with standard water containing fipronil to maintain the environmental condition in the entire experiment period. Exposure time was two weeks, and 1-week recovery period in fipronil-free water. Shrimp were taken at the end of days 1, 3, 7, 14, 15 and 21. In preparation for AChE enzyme activities, hemolymph were taken from each shrimp, and homogenized in ice-cold 50mM Tris-HCl buffer. The homogenate was cen-

trifuged at 10,000xg(for 20 min at 4^0C). 1000μL working solution of acetylcholinesterase kit(Cat. No. 1.12131, Merck) were added to the 10μL sample and it was read in a spectrophotometer for 3 min at a wavelength of 405 nm. AChE activities of hemolymph were expressed kU/I= (ΔA/min)×15.2.

Statistical differences in AChE enzyme activities at various fipronil concentrations were tested by one-way analysis of variance(ANOVA). A level of $p<0.05$ was determined to be statistically significant.

RESULTS AND DISCUSSION

According to the static renewal method for acute toxicity testing[2], median lethal concentrations(LC_{50}) of fipronil for freshwater shrimp (*Caridina japonica*) were measured. The calculated 48h-LC_{50}, 72h-LC_{50}, and 96h-LC_{50} values are list in TABLE 1.

From our investigation, the longer the time of exposure, the longer the toxic effect of fipronil lasted. The toxicity of fipronil to invertebrates were relatively high, 96-h LC_{50} of fipronil with freshwater shrimp(*Caridina japonica*) was 0.086μg/L, and with *Palaemonetes pugio*, *Macrobrachium nipponese*, *Eriocheir sinensis*, and *Procambarus clarkia* were 0.32, 4.32, 8.56, and 63.7μg/L, respectively[8,14]. *C.japonica* was more sensitive to fipronil in other tested organisms.

Physicochemical factors(temperature, pH, and dissolved oxygen) were measured throughout each sublethal test(TABLE 2). All Physicochemical parameters remained constant throughout the experimental period.

Concentrations used in the acetylcholinesterase (AChE) activities of fipronil on *C.japonica* were equivalent to approximately 12%, 23%, and 46% of the 96-h LC_{50} value according to acute toxicity study. No mortality was recorded during the three weeks of the experiment for all exposure concentrations studied. The results of AChE activity analyses are presented in figure 1. With the exception of 21-d (recovery period), all sampling intervals were found to statistically significantly($p<0.05$) differ between the highest(4.0 μg/L) exposed groups and the untreated groups. Specially, a decrease in AChE activity in exposed *C.japonica*, a maximum decrease of 60% was observed at the 3-d exposure time at the same exposed groups.

TABLE 1 : Fipronil concentrations and freshwater shrimp (*Caridina japonica*) mortality(%) in acute toxicity and median lethal concentration(LC_{50})

Nominal concentration (μg/L)	Mean mortality (%)	LC_{50}(μg/L)
0.02	0	0.086 (96h)
0.05	20	(0.061-0.121)
0.10	60	
0.20	90	
0.40	100	
0.05	0	0.165 (72h)
0.10	30	(0.099-0.272)
0.20	60	
0.4	80	
0.6	100	
0.1	0	0.399 (48h)
0.2	10	(0.263-0.608)
0.4	50	
0.6	100	

The 95% confidence limits are given in parentheses

TABLE 2 : Physicochemical parameters monitored over the experimental period

Parameter	Control	0.01μg/L	0.02μg/L	0.03μg/L
Temperature(^{0}C)	23.9±0.5	24.2±0.4	24.1±0.3	23.8±0.4
pH	7.5±0.41	7.7±0.49	7.7±0.61	7.6±0.58
Dissolved oxygen(mg/l)	7.2±0.46	7.3±0.29	7.4±0.27	7.3±0.38

All values are given as the mean±SD; n=18

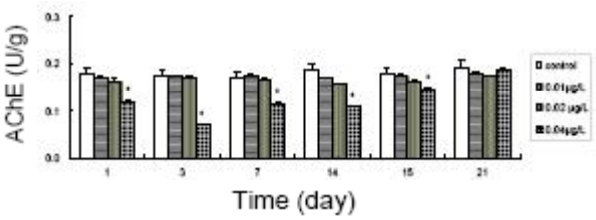

Figure 1 : Effect of fipronil on the acetylcholinesterase activity(U/g) of freshwater shrimp(*Caridina japonica*) during the experimental period (mean±SD, n=3). Exposure time was two weeks, and one week recovery period in fipronil-free water; *Indicates a significant difference compared to the control group ($p<0.05$)

Reductions in acetylcholinesterase(AChE) activities of *C.japonica* were correlated to fipronil concentrations at the highest sublethal levels (4.0μg/L). There were no differences in AChE activities among 0.01μg/L, 0.02μg/L, and control groups. Almost no toxic effect was seen at 0.02μg/L fipronil which is equivalent to 23% of the 96-h LC_{50} value in this study. Less than 0.02μg /L is proposed as a biologically safe concentration which can be used for establishing tentative water quality criteria concerning of same size *C.japonica*. And

there are considerable studies reporting AChE effects after fipronil exposure have mainly with aquatic animals[13,8,14,11]. Those studies also concluded that analysis of AChE levels in aquatic animals would be a useful indicator of fipronil exposure.

CONCLUSIONS

There has been considerable research on the physiological, biochemical, and molecular aspects of insecticides absorption, distribution, biotransformation, and excretion in mammals. Much more extensive biochemical toxicological research in mammals than in aquatic animals, it is clear that there is considerable overlap in many of the basic aspects of these processes. In addition, many of the most effective insecticides in current use act on the insect nervous system[16,12]. In the present study, the correlation between AChE activity and fipronil toxicity in *C.japonica* should be recommended as a potential indicator species of relative insecticides exposure.

REFERENCES

[1] A.G.Heath; 'Water Pollution and Fish Physiology', CRC Press, Boca Raton, FL, **(1987)**.

[2] A.L.Buikema (Jr.), B.R.Niederlehner, J.Cairns (Jr.); Water Res., **16**, 239-262 **(1982)**.

[3] D.Hainzl, J.E.Casida; Proc.Natl.Acad.Sci.USA, **93**, 12764-12767 **(1996)**.

[4] D.Hainzl, L.M.Cole, J.E.Casida; Chem.Res. Toxicol., **11**, 1529-1535 **(1998)**.

[5] D.B.Gant, A.Chalmers, M.Wolff, H.Hoffman, D. Bushey; Rev.Toxicol., **2**, 147-156 **(1998)**.

[6] D.F.Boesch, R.E.Turner; Estuaries, **7**, 460-468 **(1984)**.

[7] D.J.Finney; 'Probit Analysis', Cambridge University Press; London, **(1971)**.

[8] D.Schlenk, D.Huggett, J.Allgood, E.Bennett, J.Rimoldi, A.Beeler, D.Block, A.Holder, R.Hovinga, P.Bedient; Arch.Environ.Contam.Toxicol., 41, 325-332 **(2001)**.

[9] H. kleerekoper; Res.Bd.Can., **33**, 2036-2047 **(1976)**.

[10] J.L.Yang, H.C.Chen; Chemosphere, **53**, 877-882 **(2003)**.

[11] J.P.Overmyer, B.N.Mason, K.L.Armbrust; Bull.Environ.Contam.Toxicol., **74**, 872-879 **(2005)**.

[12] J.R.Bloomquist; Rev.Entomol., **1**, 163-190 **(1996)**.

[13] M.Heinz, H.Olaf, M.Norbert; Parrsitol.Res., **87**, 198-207 **(2001)**.

[14] P.B.Key, K.W.Chung, A.D.Opatkiewicz, E.F.Wirth, M.H.Fulton; Bull.Environ. Contam.Toxicol., **70**, 533-540 **(2003)**.

[15] P.J.Den Besten, S.Valk, E.Weerlee, R.Nolting, J. Postma, J.Everaarts; Mar.Environ.Res., **51**, 365-387 **(2001)**.

[16] T.Narahashi; Pharmacol.Toxicol., **70**, 1-14 **(1996)**.