Bull Environ Contam Toxicol (2012) 88:25–29
DOI 10.1007/s00128-011-0431-8

Effects of Chlorpyrifos Exposure on Growth and Food Utilization in Australian Catfish, *Tandanus tandanus*

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Received: 13 July 2010 / Accepted: 11 October 2011 / Published online: 20 October 2011

Abstract Australian catfish, *T. tandanus* were exposed to a short term pulse of chlorpyrifos at 2 or 10 μg L^{-1} and grown in optimal conditions to investigate the effect of the pesticide on fish growth. The final weight was lower in fish exposed to chlorpyrifos (8.54 ± 0.19 and 8.77 ± 0.44 g) respectively compared to the control fish (10.5 ± 0.72 g) while the hepatosomatic index in fish exposed to chlorpyrifos was a higher (1.86 ± 0.10 and 2.01 ± 0.12) than in the control fish (1.65 ± 0.14). Both bi-weekly growth rate and brain Acetylcholinesterase (AChE) activity increased with post-exposure time.

Keywords Chlorpyrifos and fish growth · Australian catfish · *Tandanus tandanus*

Organophosphate (OP) pesticides are widely used in tropical agriculture (Chandrasekara and Pathiratne 2007; Sun and Chen 2008). These pesticides are likely to enter water bodies in several ways, including spray drift, leaching from soil and water, and run off from agriculture (Chandrasekara and Pathiratne 2007). The use of these chemicals may have significant impacts on many non-target aquatic species, including fish, which are particularly sensitive to OP toxicants (Hai et al. 1997). Although effects of OP pesticides on biological functions of fish have been widely studied, information on the effects of these pesticides on growth of fish is very limited. Comprehensive studies were conducted on various effects of OP exposure in fish, including inhibition in Acetylcholinesterase (AChE) activity (Sancho et al. 1997; Gül 2005; Chandrasekara and Pathiratne 2007; Kavitha and Rao 2008); and alteration in metabolism (Lal and Singh 1986; Hai et al. 1997; Tripathi et al. 2003; Kavitha and Rao 2008). In a review by Woltering (1984), it was noted that negative effects of OP exposure on fish growth were observed by Jarvinen and Tanner (1982) in fathead minnow juveniles, *pimephales promelas*; by Cleveland and Hamilton (1983) in rainbow trout, *Salmo gairdneri* and channel catfish, *Ictalurus punctatus*; by Nagel et al. (1991) in zebrafish, *Brachydanio rerio*. The most comprehensive study on the effects of OP exposure on growth of fish was only found recently in the study by Cong et al. (2009) on snakehead fish, *Channa striata.*

However, there are no published studies on the impact of OP pesticide exposure on nutrient assimilation in fish, which is especially relevant to fish production of those species that are important to aquaculture. In Australia, no study has been conducted on the effects of pesticides on the Australian catfish, *Tandanus tandanus*. This study evaluated the effects of a pulse exposure of chlorpyrifos followed by subsequent recovery in optimum conditions on the growth and food utilization of Australian catfish, *Tandanus tandanus*, simulating field pesticide exposure conditions. This study also measured AChE activity of the fish following the pulse exposure to chlorpyrifos in order to evaluate the effects of chlorpyrifos on both AChE and growth during the recovery period.

Materials and Methods

Experimental fish were procured from Namoi Valley Aquafarming Pty. Ltd., New South Wales, Australia, and acclimated for 1 month in 2,000 L flow-through fiberglass tanks before being used for experiments. All experimental

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procedures were conducted in accordance with RMIT Animal Ethics Permit AEC0614. The experiment consisted of 3 treatments with 4 replicates per treatment, i.e. control, 2 μg L^{-1} laboratory grade chlorpyrifos (Sigma Aldrich Pty Ltd) and 10 μg L^{-1} chlorpyrifos, henceforth referred to as the control, treatment 1 and treatment 2, respectively. The exposure concentrations were selected in reference to the exposure concentrations used by Chandrasekara and Pathiratne (2007) on Nile tilapia, *Oreochromis niloticus*. These authors exposed Nile tilapia to 0.5–12 μg L^{-1} of chlorpyrifos, and significant effects on AChE activity of fingerlings were observed only at exposure of 8 and 12 μg L^{-1} chlorpyrifos. In addition, AChE activities of fish exposed to these concentrations was not significantly different from one other. Therefore, it might be expected that Australian catfish fingerlings of similar size would be affected similarly when exposed to a concentration >8 μg L^{-1} chlorpyrifos. Therefore, 10 μg L^{-1} was chosen for the high treatment. In each treatment, 1 tank was randomly reserved to sample fish for AChE activity analysis and the remaining 3 tanks were reserved for the growth experiment. At the start of the experiment, 120 fish were randomly stocked into twelve tanks (36 cm × 50 cm × 30 cm) which had been assigned to the 3 treatments. Fish in treatment 2 were transferred to clean water after 2 h exposure and sampled for AChE analysis. These fish were distressed and demonstrated abnormal behaviour, including lying at the bottom, unbalanced movement, pale colour and exhausted breathing at the time of transfer. In treatment 1, fish were transferred to clean water after 22-h exposure, when 1 fish was found dead. The ten fish from one tank of the control and treatment 1 were sampled for AChE analysis at this time. Experimental fish in the remaining tanks were then transferred randomly to clean water in tanks (10 fish per tank) in a flow-through water system and fed twice daily to satiation with a laboratory formulated optimal fishmeal-based diet, for 6 weeks, to evaluate the effect of chlorpyrifos on the growth and nutrient assimilation of the fish.

Ten fish were sampled at the start of the experiment for fish carcass analysis. At the end of the experiment, 5 fish from each tank were sampled for analysis of AChE activity and liver weight measurement and the other 5 fish were sampled for fish carcass analysis. Fish were weighed at the start of the experiment and then every 2 weeks during the 6 week experimental period, in order to adjust food provided to fish as fish body weight altered. Fish were fed to satiation, twice daily between 9.00–9.30 and 17.00–17.30, except the days before weighing when fish were fed once. The remaining food was siphoned, dried and recorded to allow for food-intake calculations. In order to account for the stability of different diets, a correction factor was calculated for each treatment. A predetermined amount (2 g) of each diet was placed in an experimental tank without fish for 30 min, the remaining food were siphoned, dried and weighed. Correction factors were calculated as follow:

$$C = ((F1 - F2) \times 100)/F1$$

where C was correction factor, F1was dry weight of food at the beginning, and F2 was dry weight of food after 30 min

Final weight, food intake (% body weight per day), percentage weight gain (%WG), food conversion ratio (FCR), protein efficiency ratio (PER), and hepatosomatic index (HSI) were used as indices to evaluate the effect of exposure to the pesticide on growth and nutrient utilization of the fish. These parameters were calculated as follows:

$$\text{Food intake}(\%\,\text{BW day}^{-1}) = 100 \times I/[(W_0 + W_t)/2 \times t$$
$$FCR = I/(W_t - W_0)$$
$$PER = (W_t - W_0) \times 100/I \times CPr_f$$
$$\%WG = (W_t - W_0) \times 100/W_0$$
$$HSI = \text{liver weight} \times 100/BW$$

where I (g) is total dry weight of food provided, W_0 (g) is total initial body weight and W_t (g) total final body weight; t (days) is duration of the fooding trial; N_t is number of fish at the end of the trial and N_0 at the start of the trial; CPr_f (%) is protein content in food, CPr_t (%) is protein content in carcass at the end of the trial; These calculations were adapted from Wang et al. (2006).

Proximate analyses of food ingredients, experimental diets, and whole fish carcasses were conducted in duplicate based on the procedures of the AOAC (1990). The AChE sample collection method was adapted from Cong et al. (2006) and Kavitha and Rao (2008). AChE activity in brain tissues was measured following the method of Ellman et al. (1961) with minor modifications as adapted by Kavitha and Rao (2008). The actual exposure concentrations of pesticide were determined using the Abraxis OP/Carbamate Kit which is based on a modification of the inhibition of the enzyme AChE by the test compound.

Dissolved oxygen (DO), pH and temperature of tank water were measured every other day using a TPS WP-81 pH meter, TPS WP-91 DO, and temperature meters while NH_3 and NO_2 were measured following the standard methods of Boyd and Tucker (1992). The average DO was 6.97 ± 0.17 mg L^{-1}, average pH was 6.81 ± 0.04, average temperature was 25.0 ± 0.16 degrees Celsius, average NH_3 concentration was 0.12 ± 0.01, and average NO_2 concentration was 0.011 ± 0.003.

Statistical analyses were conducted using SPSS for Windows software (version 16.0). The Levene's test was used to test homogeneity of variances. Since there were no differences in replicates within treatment, one way analysis of variance (ANOVA) was used to test the statistical

Table 1 Nominal and actual exposure concentrations of chlorpyrifos

	Concentrations (μg L^{-1} of chlorpyrifos)	
Nominal concentrations	2	10
Actual concentration at start	2.32 ± 0.02	11.7 ± 0.17
Actual concentration at end of exposure	2.37 ± 0.02	Not determined

Table 2 Brain AChE activity of Australian catfish (μM min^{-1} g^{-1}) after exposure to 2 and 10 μg L^{-1} chlorpyrifos and 6 weeks after transfer to clean water

Time point	Exposure concentrations of chlorpyrifos		
	0	2 μg L^{-1} for 22 h	10 μg L^{-1} for 2 h
After exposure	3.62^{a} ± 0.30	0.57^{b} ± 0.11	1.24^{b} ± 0.10
After 6 weeks	1.96^{a} ± 0.10	1.37^{b} ± 0.07	1.43^{b} ± 0.07

Values are expressed as mean ± SE (n = 5); in the same rows, AChE activity values indicated with same superscript are not significantly different ($p > 0.05$)

significance of the differences between the experimental treatments. The LSD post hoc test was performed to test the differences between the means at $p < 0.05$. Data in percentage were transformed using arc sine of the square root of the value.

Results and Discussion

The actual concentrations of chlorpyrifos (Table 1) were close to the nominal concentrations and were stable during the exposure period. This ensured that the pesticide was effective during the exposure

The AChE activities of fish after exposure to both selected concentrations (Table 2) were significantly different from that of the control fish ($p < 0.05$), although AChE activity of fish recovered during the experiment. Although the exposure time for treatment 2 was only 2 h, it was 22 h for treatment 1 making it irrelevant to compare the two treatments, comparisons between AChE activities of fish at the two time points are important since the exposed fish were randomly redistributed in the 3 replicate tanks for grow-out, following each exposures.

Final weight of the fish in both treatments (Table 3) was significantly poorer than that of the control fish ($p < 0.05$). The poor growth of fish could be the result of AChE inhibition as reported in previous studies, although these studies only emphasized the toxicity of the pesticide

Table 3 Growth and nutrient utilization of Australian catfish after 6 weeks in optimal conditions following short pulse exposure to chlorpyrifos

Parameters	Treatments		
	Control	2 μg L^{-1} for 22 h	10 μg L^{-1} for 2 h
Initial weight (g)	4.75 ± 0.09	4.65 ± 0.03	4.69 ± 0.13
Final weight (g)	10.5^{a} ± 0.72	8.54^{b} ± 0.19	8.77^{b} ± 0.44
Food intake (% BW day^{-1})	2.93 ± 0.07	2.90 ± 0.04	2.95 ± 0.03
FCR	1.65^{a} ± 0.13	2.06^{b} ± 0.06	2.06^{b} ± 0.10
PER	1.87^{a} ± 0.15	1.48^{b} ± 0.05	1.49^{b} ± 0.07
HSI	1.65^{a} ± 0.14	1.86^{ab} ± 0.10	2.01^{b} ± 0.12

Values are expressed as mean ± SE (n = 3, replicate/treatment); in the same rows, values indicated with same superscript are not significantly different ($p > 0.05$)

(Jarvinen and Tanner 1982; Cleveland and Hamilton 1983; Woltering 1984; Nagel et al. 1991). Recently, Cong et al. (2009) found a significant impact of diazinon exposure on the growth of snakehead fish (*Channa striata*). It must be noted, however, that the exposure method used in the present study was different from that used in the previous studies of Nagel et al. (1991) and Cong et al. (2009). Many of the previous studies focused on the effects of the toxicant under very stressful conditions, in which fish were in continuous exposures to pesticides for longer periods, such as 4 days in the study by Jarvinen and Tanner (1982) on fathead minnows, 42 days in the study by Nagel et al. (1991) on zebra fish, and 4-day exposures repeated for 4 times in the study by Cong et al. (2009). The short pulse exposure applied in the present study is a somewhat more accurate simulation of the actual pesticide contamination conditions in aquaculture farms, where pesticides are sprayed once, contaminated water remains for a short period of time and is subject to water exchange.

FCR of fish exposed to both concentrations of chlorpyrifos was statistically different from that of the control fish ($p < 0.05$). Similar effects of chlorpyrifos exposure on PER of fish can be seen in data presented in the same table (Table 3). FCR measures the amount of food needed to produce a unit weight gain. Since food intake of fish was not significant different among treatments, the poor FCR of OP exposed fish may be attributed to their significantly poorer ability to utilize food for growth compared to that of the control fish. Similarly, the PER measures the weight gain of fish per unit of protein intake. Since all experimental fish were fed with the same food and had similar food intake, the poorer PER of the exposed fish may also be attributed to the poor protein utilization of the fish. The poor FCR and PER of the exposed fish also suggests that

fish exposed to chlorpyrifos utilized food and nutrient less efficiently compared to the control fish.

Chlorpyrifos exposure significantly affected the HSI of fish (Table 3). HSI of fish increased with the increase in exposure concentrations. HSI of fish exposed to 10 μg L^{-1} chlorpyrifos was significantly greater than that of the control fish ($p < 0.05$). As the main organ for detoxification, the liver is affected when fish are exposed to pesticides (Sancho et al. 1997; Velmurugan et al. 2007). The effects of exposure to pesticides on morphology and biochemical properties of liver in fish have been reported in many previous studies (Gill et al. 1991; Gimeno et al. 1994; Sancho et al. 1997; Rao 2006; Velmurugan et al. 2007). In the present study, exposure to chlorpyrifos significantly increased the HSI of the exposed fish, especially at the concentration of 10 μg L^{-1}. Although HSI of fish exposed to 2 μg L^{-1} chlorpyrifos was not significantly greater than that of the control fish, there was a trend for an increase in HSI with increase in exposed pesticide concentration. This is probably a result of the roles of liver in the pesticide detoxification process and the enlargement of the liver may well be a result of increased liver activity. This particular effect has also been reported in many other studies evaluating different types of pesticides. Sancho et al. (1997) found a significantly increased HSI in eel exposed to fenitrothion. Similarly, Gill et al. (1991) found a significant elevation of HSI in the freshwater fish *Barbus conchonius* after exposure to endosulfan for a prolonged period. The same impact was also reported in *Anguilla anguilla* by Holmberg et al. (1972; cited by Sancho et al. 1997) and in the rainbow trout, *S. gairdneri* by Lidman et al. (1976; cited by Gill et al. 1991). Gill et al. (1991) also correlated the enlargement of fish liver to an increase in liver protein and total lipid.

The growth recovery of experimental fish during the 6-week growth experiment after chlorpyrifos exposure is demonstrated in Table 4. In the first 4 weeks of the experiment, the growth rate of exposed fish in both concentrations was significantly lower than that of the control fish ($p < 0.05$) though a slight improvement was seen during weeks 3 and 4. The growth rate of exposed fish was increased to a rate similar to that of the control fish ($p > 0.05$) during the last 2 weeks of the recovery period. Therefore, it was apparent that chlorpyrifos mainly impaired the growth of Australian catfish during the first 4 weeks after exposure, severely in the first 2 weeks. Although growth of the exposed fish was comparable to that of the control fish after 4 weeks of the experiment, growth of the exposed fish at week 4 was still lower than that of the control fish and was still increasing during the last 2 weeks of the experiment. However, since the exposed fish grew very slowly at the beginning of the experiment, the overall growth performance of the fish exposed to both concentrations of chlorpyrifos was significantly poorer than that of the control fish. The results revealed that the fish were able to recover, since their growth rate was as high as the control during the last 2 weeks of the experiment. It is, therefore, possible that the poor early growth performance would be compensated for and the fish might recover if favorable conditions were prolonged. This implies the importance to fish growth of a reduction in repeated spraying of OP pesticides in the field.

Table 4 Fortnightly percentage weight gain of fish during 6-week feeding experiment after 22-h exposure to different concentrations of chlorpyrifos

Time	Treatments		
	Control	2 μg L^{-1} for 22 h	10 μg L^{-1} for 2 h
Start–week 2	$32.2^{a} \pm 1.34$	$16.1^{b} \pm 3.29$	$18.7^{b} \pm 1.93$
Week 2–week 4	$27.7^{a} \pm 2.93$	$21.1^{b} \pm 1.42$	$20.4^{b} \pm 1.86$
Week 4–week 6	$30.5^{a} \pm 2.27$	$30.9^{a} \pm 2.29$	$30.7^{a} \pm 0.75$

Values are expressed as mean ± SE (n = 3, replicate/treatment); in the same rows, values indicated with same superscript are not significantly different ($p > 0.05$)

The design of this study clearly demonstrates that when fish are exposed to toxicants as a short pulse at a higher exposure concentration the subsequent long term effects could very realistically mimic effects of exposure to a lower concentration for a longer period, which often occurs in field conditions. This experiment has clearly demonstrated this, at least for fish exposed to chlorpyrifos.

Acknowledgments The authors thank the Vietnamese Overseas Scholarship Program (Project 322) for sponsorship of the PhD scholarship given to the first author. We are also grateful to RMIT Research and Innovation for partial financial support to the first author. We thank James Oliver for technical support during the experiments. We express our special appreciation of contribution of raw materials for the research by Ridley Aquafeed Australia and Riverland Oilseed Processors.

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