Acute Toxic Effects of Chlorpyrifos-trichlorphon Mixture and Acetochlor on Bufo Gargarizans Tadpoles

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Abstract: The acute toxic effects of two pesticides on Bufo gargarizans tadpoles were investigated in order to provide a scientific basis for the monitoring of aquatic environments and proper use of pesticides. Experimental methods designed to determine the acute effects of specific agents on aquatic organisms were used to explore acute toxic effects of single exposure of chlorpyrifos-trichlorphon mixture and acetochlor on Bufo gargarizans tadpoles. For chlorpyrifos-trichlorphon mixture, at 24, 48, 72, and 96 h, median lethal concentrations (LC50) for Bufo gargarizans tadpoles were 12.023, 7.499, 4.977, and 2.799 μl/L, while for acetochlor were 0.064, 0.049, 0.043, and 0.026 mg/L. The safe concentrations (SC) were 0.875 μl/L and 0.009 μl/L for chlorpyrifos- trichlorphon mixture and acetochlor, respectively. These results show that the toxicity of acetochlor against B. gargarizans tadpoles was more toxic than that of chlorpyrifos- trichlorphon mixture, and it will provide a basis for proper use of these two pesticides.

Keywords: Chlorpyrifos-trichlorphon Mixture; Acetochlor; Bufo Gargarizans Tadpoles; Acute Toxicity

1. Introduction

Biodiversity is declining at worldwide scale, and amphibians represent the prime example of the modern biodiversity crisis since they are the most threatened and rapidly decline vertebrate group, disappearing from different habitats on a global scale (Stuart et al., 2004; Pounds et al., 2006; Brühl et al., 2013). Pesticide use, already considered one of the important factors in the decline of the amphibian species (Collins and Storfer et al., 2003; Hayes et al., 2006; Brühl et al., 2013). China produces and uses more pesticide than any other country in the world. Pesticides play an extremely important role in controlling agricultural pests and diseases and in increasing crop yield. However, improper use can cause serious damage to aquatic environments due to the high toxicity and high rates of retention and accumulation of these agents (Hu et al., 2008). This threatens amphibians and other aquatic organisms. During the larval (tadpole) stage, tailless amphibians live in aquatic environments. Their skin lacks protective structures and is highly permeable. When amphibians' habitats are polluted with pesticides, tadpole mortality increases and physiological defects may appear (Li et al., 2010; Lin et al., 2009). In recent years, the decline of amphibian populations worldwide has become more and more serious. Studies have shown that, for amphibians that live near farmland, environmental pollution caused by improper use of pesticides is one of the most important causes of this phenomenon (Wu et al., 2004).

Amphibian tadpoles are suitable for use as biological indicators for monitoring aquatic environment pollution. In recent years, there have been a large number of reports on the impact of agricultural pollutants on amphibians in China and other countries (Li et al., 2010; Lin et al., 2009; Wu et al., 2004; Brühl et al., 2013). These studies have significant implications for environmental protection, pollutant management, and proper use of pesticides. Bufo gargarizans belongs to family Bufonidae, Order Anura, Class Amphibia, and is distributed in an extremely wide range in China (Lin et al., 2009; Yan et al., 2009). It serves as an experimental material in many scientific studies. It can also be used as a biological indicator of water pollution. In this way, in the present study, the toxic effects of two pesticides that are widely used in farmlands in southwest China, chlorpyrifos-trichlorphon mixture and acetochlor, on Bufo gargarizans were investigated. These findings may provide a basis for improving environmental protection and facilitating proper use of pesticides.

2. Materials and Methods

2.1. Experimental Materials

The chlorpyrifos-trichlorphon mixture (Chemical formula: C9H11Cl3NO3PS / C4H8Cl3O4P, m. w. 350.5/257.45 respectively)used in the experiment contained commercial 40% emulsifiers for combined pesticides (EC) including 20% chlorpyrifos and 20% trichlorphon, manufactured by Zhongjin Chemical Co., Ltd. (Shanxi, China). It was now widely used to kill the insect pests in farming, forestry, and environment. Acetochlor (90%, EC), was obtained from Nongken Pesticide Chemical Co., Ltd. (Jiangsu, China) (Chemical formula : C14H20ClNO2, m. w. 269.77). Acetochlor is a widely used herbicide in China and is mainly used to control a wide range of annual grasses and some broadleaf weeds. It can be used in seedbeds and seed transplant fields as well as in some crop fields such as wheat, barley, beet, cotton rape, vegetables and peanut (Geng et al., 2005a).

The experiment was conducted during mid to late April, 2012. Bufo gargarizans were collected from farmland ditches in Qingyuan Village, Yucheng District, City of Ya'an, Sichuan, China. Prior to the experiments, tadpoles were maintained in buckets of tap water that had been aerated for one day before tadpole placement. In total, undamaged tadpoles (Stage 26th) of the same body size were used in the experiment (Zhao et al., 1991). These were maintained in buckets in the laboratory for three days to allow the tadpoles to fully adapt to the laboratory environment (Zhou et al., 1989). One day before the experiment, feeding was stopped, tadpoles were monitored and dead individuals were cleared out in a timely manner.

2.2. Experimental Methods

(1) Preliminary experiments

The 96 h hydrostatic method was used (Peng et al., 2009). A set of chlorpyrifos-trichlorphon mixture and acetochlor concentrations with rather large concentration spacing was used. At 24, 48, 72, and 96 h, the status and mortality of Bufo gargarizans were observed. Dead individuals were promptly removed, and the mortality rate was calculated. Based on these data, minimum absolute lethal concentration at 24 h (24 h LC100) and maximum zero-death concentration at 96 h (96 h LC0) were determined. These were used as references for the main experiment. The criteria used to determine tadpole death were as follows: no reaction when the head and tail of the tadpole were touched with a glass rod; and no response after the tadpole was placed in toxin-free water for 5 min (Zhang et al., 2007).

(2) Acute toxicity testing

The acute toxicity test is quick to perform and can indicate the strength of the toxins (Peng et al., 2009). In this way, in the main experiment, standard methods of testing acute toxic effects of toxic substances on aquatic organisms were used. According to the results of preliminary experiment, in the range between LC0 and LC100, a set of arithmetic concentration gradients was established for chlorpyrifos- trichlorphon mixture and acetochlor. For chlorpyrifos-trichlorphon mixture, 5 concentration groups, 5, 10, 15, 20, and 25 μl/L and a blank control group were established. For acetochlor, 5 concentration groups, 0.04, 0.05, 0.06, 0.07, and 0.08 μl/L, were established. Three replicates were performed per concentration group. There were 30 tadpoles per bucket.

During the experiment, which lasted 96 h in total, experimental solutions were changed every 24 h. Toxic reactions and deaths of tadpoles were monitored and recorded.

2.3. Data analysis

SPSS 16.0 (SPSS, Microsoft, Redmond, WA, U.S.) was used for data analysis. The specific steps were as follows. First, the Probit method was used to calculate LC50s at 24, 48, 72, and 96 h, respectively. Here, LC50 is the concentration corresponding to Prob. 0.5. SC was calculated using the formula SC = 48 h LC50*0.3/(24 h LC50/48 h LC50)2(Zhou et al., 1989). Next, concentrations of isoprothiolane and dimethoate were log transformed. The cumulative relative mortality of the tadpoles was transformed into cumulative probability of death. Then linear regression was performed on log concentration and the probability of death was calculated to produce the toxicity regression equation. P<0.05 was considered statistically significant; P<0.01 was considered to be of prominent statistical significance.

3. Results

The acute toxic effects of chlorpyrifos-trichlorphon mixture and acetochlor on Bufo gargarizans are shown in Table 1. It can be seen that during the same time period, tadpole mortality increased as chlorpyrifos-trichlorphon mixture/acetochlor. concentration increased. This suggests that within certain concentration range, chlorpyrifos-trichlorphon mixture and acetochlor have acute toxic effect on Bufo gargarizans. In addition, for the same concentration of same pesticide, mortality tended to increase the exposure duration increased. This suggests that the two pesticides have cumulative effects on Bufo gargarizans.

Analysis of the data shown in Table 1 was performed using SPSS 16.0. Results showed that, for Bufo gargarizans, LC50s of chlorpyrifos-trichlorphon mixture at 24 h, 48 h, 72 h, and 96 h were 12.023, 7.499, 4.977, and 2.799μl/L, respectively. SC was 0.875 μl/L. The LC50s of acetochlor at 24 h, 48 h, 72 h, and 96 h were 0.064, 0.049, 0.043, and 0.026 μl/L, respectively. SC was 0.009μl/L (Table 2). Regression equations and correlation coefficients of chlorpyrifos-trichlorphon mixture or acetochlor and tadpole death probability obtained from linear regression analysis are shown in Table 2. As illustrated in Table 2, there was a significant linear correlation between the probability of tadpole death and the logarithm of pesticide concentration ($P<0.05$). Results on LC50s suggest that the toxic effects of acetochlor on Bufo gargarizans are stronger than those of chlorpyrifos-trichlorphon mixture. In addition, SCs of chlorpyrifos-trichlorphon mixture and acetochlor were 0.875 μl/L and 0.009 μl/L, respectively, also suggesting that the influence of acetochlor on environment is more pronounced than that of chlorpyrifos-trichlorphon mixture.

4. Discussion

Due to the speed which with amphibians can be bred using artificial means, their short life cycle, the ease of caring for and observing them, and their extreme sensitivity to water-borne toxins during embryonic development, amphibians can be ideal for toxicology studies(Peng et al., 2009; Yao et al., 2005; He et al., 2007). They have been used as biological indictors in evaluations of water pollution (Xu et al, 2003). The tadpoles of Bufo gargarizans have also been used as experimental materials in many toxicity studies (Liu et al., 2008; Zhang et al., 2006).

Regression analysis showed there to be a positive linear correlation between the probability of death among Bufo gargarizans and logarithms of pesticide concentration, and the R values of all linear regression equations were above 0.8 (Table 2). In addition, the b value in linear regression equation of logarithm of pesticide concentration and probability of tadpole death represents the slope. As the independent variable (x) changes one unit, the dependent variable (y) changes an average of b units. In the present study, as the logarithm of pesticide concentration increased by one unit, the probability of tadpole death increased by b units. In this way, b represents the degree of heterogeneity of the sensitivity of biological populations to different pesticides. The smaller the b value, the greater the heterogeneity of the tadpole population. Specifically, the less uniform the responses of tadpole population, the less sensitive the tadpoles were to pesticides (Zhang et al., 2009; Geng et al., 2005b; Xue et al., 2005). As shown in Table 2, the b value in the regression equation of acetochlor concentration and tadpole cumulative mortality is notably larger than that of the regression equation of chlorpyrifos-trichlorphon mixture concentration and tadpole cumulative mortality. This suggests that, for a given environment, increases in the concentration of acetochlor cause more damage than increase in the concentration of chlorpyrifos-trichlorphon mixture and that Bufo gargarizans are more sensitive to acetochlor than to the latter.

However, this study was performed in a laboratory environment, which is different from the tadpoles' natural environment, on which many influencing factors act at the same time. Different pollutants in the water may mix with chlorpyrifos-trichlorphon mixture or acetochlor and enhance their toxic effects on the organisms that live there, possibly through synergistic effects. For example, at low concentrations, imidacloprid and trichlorfon do not have toxic effects on Bufo gargarizans tadpoles, but when they are both present in the same environment, they can greatly enhance each other's toxic effects (Lu et al., 2002). In addition, acute toxicity test results usually do not reflect the long-term effects of environmental pollutants on aquatic organisms. Although our data can reflect the toxicity of chlorpyrifos-trichlorphon mixture and acetochlor to a certain extent, the manner in which they affect real ecosystems still merits further investigation that might provide further data and a foundation for the protection of amphibians and other organisms.

In addition to directly causing death, the toxic effects of pesticides on amphibians may also manifest by affecting animal behavior, decreasing the birth rate, blocking endocrine agents, and inducing immune inhibition. For example, some common pesticides (Lu et al., 2002) can induce embryonic malformation and tadpole death in Rana nigromaculate, and some herbicides can cause erythrocyte micronucleus and nuclear abnormalities in Bufo gargarizans tadpoles.

In reality, improper use of pesticides will inevitably severely affect amphibian tadpoles. In this way, the present study provides reference material for the practical use of chlorpyrifos-trichlorphon mixture and acetochlor. It also demonstrates the value of using amphibians for biological monitoring. In these ways, the results of this study may play a role in scientific and standardized use of pesticides, protection of aquatic environments, and studies of the growth, development, and reproduction of amphibians living in bodies of water near farmland.

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Table 1. Acute Toxic Effects of Chlorpyrifos-trichlorfon Mixture and Acetochlor on Bufo Gargarizans Tadpoles

Pesticide Name	Pesticide Concentration	Number of Tadpoles	Cumulative Percent Mortality

	μl/L	N	24 h	48 h	72h	96 h
chlorpyrifos- trichlorphon mixture	Control group	30	0	0	0	0
	5	30	13.3	26.7	50	73
	10	30	36.7	60	76.7	83.3
	15	30	66.7	90	100	100
	20	30	73.3	100	100	100
	25	30	83.3	100	100	100
acetochlor	Control group	30	0	0	0	0
	0.04	30	10	16.7	33.3	66.7
	0.05	30	3.87	56.7	66.7	100
	0.06	30	33.3	73.3	100	100
	0.07	30	56.7	100	100	100
	0.08	30	83.3	100	100	100

Table 2. Linear Regression Parameters of Acute Toxic Effects of Chlorpyrifos-trichlorphon Mixture and Acetochlor Acting on Bufo Gargarizans Tadpoles

Pesticide	**Time**	**Toxicity Regression Equation**	**Correlation Coefficient**	***P***	**Median Lethal Concentration**	**Safe Con-centration**
	(h)	y = a + bx	R		LC_{50} (μl/L)	SC (μl/L)
chlorpyri-fos – trichlor-phon mixture	24 h	Y=3.101x+1.748	0.989	0.000^{**}	12.023	0.875
	48 h	Y=4.011x+1.491	0.968	0.002^{**}	7.499	
	72 h	Y=3.119x+2.827	0.925	0.009^{**}	4.977	
	96 h	Y=2.225x+4.005	0.873	0.020^{*}	2.799	
acetochlor	24 h	Y=7.411x+13.861	0.909	0.012^{*}	0.064	0.009
	48 h	Y=10.183x+18.318	0.961	0.003^{**}	0.049	
	72 h	Y=8.731x+16.950	0.871	0.020^{*}	0.043	
	96 h	Y=5.203x+12.941	0.857	0.024^{*}	0.026	

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