

Acute and joint toxicity of three agrochemicals to Chinese tiger frog (*Hoplobatrachus chinensis*) tadpoles

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Abstract: We studied acute and joint toxicity of three different agrochemicals (chlorantraniliprole, flubendiamide-abamectin and penoxsulam) to Chinese tiger frog (*Hoplobatrachus chinensis*) tadpoles with the method of stability water tests. Results showed that the three agrochemicals increased tadpole mortality. For acute toxicity, the LC₅₀ values after 24, 48 and 72 h of chlorantraniliprole, flubendiamide-abamectin and penoxsulam exposure were 5.37, 4.90 and 4.68 mg/L; 0.035, 0.025 and 0.021 mg/L; 1.74, 1.45 and 1.29 mg/L, respectively. The safety concentrations (SC) of chlorantraniliprole, flubendiamide-abamectin and penoxsulam to the tadpoles were 1.23, 0.30 and 0.003 mg/L, respectively. Based on these findings, chlorantraniliprole and penoxsulam were moderately toxic, while flubendiamide-abamectin was highly toxic. All pairwise joint toxicity tests showed moderate toxicity. The LC₅₀ values after 24, 48 and 72 h of exposure were 7.08, 6.61 and 6.03 mg/L for chlorantraniliprole+penoxsulam, with corresponding values of 2.455, 2.328 and 2.183 mg/L for chlorantraniliprole+flubendiamide-abamectin, and 1.132, 1.084 and 1.050 mg/L for penoxsulam+flubendiamide-abamectin, with safe concentrations of 1.73, 0.63 and 0.30 mg/L, respectively. For toxic evaluations of pairwise combinations of the three agrochemicals, only the joint toxicity of chlorantraniliprole and flubendiamide-abamectin after 24 h was found to be synergistic, whereas all other tests were antagonistic. Our findings provide valuable information on the toxic effects of agrochemicals on amphibians and how various types of agrochemicals can be reasonably used in agricultural areas.

Keywords: Agrochemical; Acute toxicity; Joint toxicity; LC₅₀; Safe concentration; *Hoplobatrachus chinensis*

Environmental degradation is one of the major causes of worldwide amphibian decline (Shuhaimi-Othman et al, 2012; Sparling & Fellers, 2009; Stuart et al, 2004; Vertucci & Corn, 1996) but is especially prominent in China, due to intensive agriculture. The influences of agriculture on the natural environment can manifest through land conservation (Hecnar, 1995), habitat fragmentation (Hayes et al, 2002) and agrochemical pollution (Blaustein et al, 2003; Davidson et al, 2002; Lavorato et al, 2013; Sparling et al, 2001). Thankfully, the wide use of agricultural chemicals and their toxicities to non-target organisms has gained increasing attention. In agricultural regions that use agrochemicals, both the redundancy and diversity of amphibians have decreased compared with those in adjacent non-agricultural regions, and even in certain areas, some species have become extinct (Bonin et al, 1997). Although agrochemicals are now generally applied within restricted areas, their application is still a

global problem, and scientists have found significant and increasing agrochemical residues in frogs since the 1990s (Datta et al, 1998; Russell et al, 1997).

Many amphibian species inhabit ponds, brooks and temporary water bodies that lay eggs within agricultural regions. Likewise, their breeding and development in late spring and early summer coincides with the agrochemical application period of highest frequency and dosage (Hayes et al, 2003; Sayim, 2008; Vertucci & Corn, 1996). However, information about the effects of agrochemicals on amphibians is still limited. Some studies have demonstrated that in agrochemical polluted habitats, amphibians

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are characterized with high mortality, high abnormality, low hatching success and small size at metamorphosis, which have induced physical problems in individual survival, e.g. liver and kidney function attenuation, deformation, and paralysis (Harfenist et al, 1989; Kamrin, 1997; Mandrillon & Saglio, 2009; Ouellet et al, 1997; Relyea, 2005). Moreover, agrochemicals have also affected the growth, development, immunoreaction and behavior of tadpoles (Bridges, 2000; Broomhall, 2005; Brunelli et al, 2009; Christin et al, 2003; Lou et al, 2013; Zhao et al, 2013).

As the most important component of vertebrate biomass, the effects of pollution have the potential to damage the balance of entire ecosystems (Sayim, 2008). Due to their easy exposure to agrochemical contaminated water and the permeability of their skins, tadpoles are considered to be a good bio-indicator of pollution (Venturmo et al, 2003).

The tiger frog (*Hoplobatrachus chinensis*) is an anuran species of the Ranidae family, inhabiting farmland and surrounding ditches (Fei et al, 2009). Due to the extensive expansion of farmland, agrochemical abuse and over hunting, the wild resources of this species have declined dramatically over the past few decades (Lin & Ji, 2005; Wang et al, 2008), and it is now classified as a Class II National Protected Species.

To detect the toxicological effects of farmland and surrounding water bodies on the tiger frog, the acute and joint toxicities of two kinds of pesticide (chlorantraniliprole and flubendiamide-abamectin) and a weedicide (penoxsulam) were analyzed. This study will provide information for the rational use of agrochemicals and the protections of amphibian species.

MATERIALS AND METHODS

Experimental agrochemicals

The following were used in this study: Chlorantraniliprole (chlorantraniliprole suspension, 200 g/L) produced by DuPont, based in the United States; Flubendiamide-abamectin (AVI-fluoroamide suspension with 10% active ingredients (6.7% flubendiamide, 3.3% abamectin)) produced by Bayer Crop Science (China), Ltd.; Penoxsulam (penoxsulam oil suspension, 25 g/L) produced by Dow Agrosciences (Indonesia) Ltd.; dechlorinated tap water (at least 48 hours), pH 6.8.

Experimental animals

Experimental tiger frog tadpoles were obtained from the breeding ponds of the Laboratory of Herpeto-Kunming Institute of Zoology (CAS), China Zoological Society

logy, Lishui University. Studied individuals were all in good health (swimming freely, with good reflexes), with homogenized size (average weight=0.03 g; average length=17.6 mm), and at G34-36 (Gosner, 1960).

Acute toxicity experiment

A few wide concentration ranges were used in the preexperiment. By observing tadpole reactions and mortalities 24 h and 48 h after the agrochemical treatments, the lethal concentration with no mortality (LC_0) and with maximum mortality (LC_{100}) were calculated and were then used as the concentration ranges in the following experiments (Xue et al, 2005).

Based on the preexperiment results, "static water changing" was used in the toxicity experiment (Zhou & Zhang, 1989). For each agrochemical, chlorantraniliprole, flubendiamide-abamectin and penoxsulam, six different concentration trials with logarithmic spacing (each trial was performed in triplicate) and one control trial were established. Totally, 10 experimental tadpoles were housed in a round 1 L plastic container with 800 mL agrochemical solution. The experimental conditions were air temperature of 29.0–29.8 °C and water temperature of 27.8–28.5 °C. During the experiment, the tadpoles were fasted and the agrochemical solutions were changed completely every 24 h, with mortalities recorded at 24, 48 and 72 h intervals. If a tadpole did not respond to pressure applied on its tail by a pair of forceps, it was considered dead (Xue et al, 2005).

Joint toxicity experiment

Based on the acute toxicity experiment results, the three agrochemicals were grouped into three pairs. For each pair, one control trial and six different concentration trials in arithmetic progression were established according to their common logarithm of concentration (each trial was conducted in triplicate) (Xue et al, 2005). Specifically, chlorantraniliprole/penoxsulam: 5.62 (4.01/1.61), 6.17 (4.41/1.76), 6.76 (4.83/1.93), 7.41 (5.29/2.12), 8.13 (5.81/2.32), 8.91 (6.36/2.55) mg/L; chlorantraniliprole/flubendiamide-abamectin: 2.04 (2.027/0.013), 2.19 (2.176/0.014), 2.34 (2.325/0.015), 2.51 (2.494/0.016), 2.69 (2.672/0.018), 2.88 (2.860/0.019) mg/L; and penoxsulam/flubendiamide-abamectin: 0.95 (0.935/0.015), 1.02 (1.004/0.016), 1.09 (1.073/0.017), 1.17 (1.151/0.019), 1.25 (1.230/0.020), 1.34 (1.319/0.021) mg/L. All other experimental settings, animals and procedures were the same as those in the acute toxicity experiment.

Statistical analysis

Mortalities of tadpoles under different agrochemical concentrations

All statistical analyses were conducted using SPSS 16.0 (SPSS inc., Chicago, IL, USA). Prior to statistical analysis, all variables were tested for normality and homogeneity. One-way ANOVA and Tukey's post hoc multiple comparisons test were used to evaluate the effects of each agrochemical (single and joint) on the mortalities of tadpoles under different concentration and different exposure time. Repeated measures ANOVA was used to evaluate the correlated effects of concentration and exposure time of each agrochemical on the mortalities of tadpoles. All results were expressed as mean±SD, with $\alpha=0.05$ taken as statistically significant.

Half lethal concentration (LC₅₀) and safe concentration (SC)

The Karber method was used to determine the LC₅₀ after 24, 48, and 72 h exposure of the individual and paired agrochemicals, respectively.

$$d=(X_K-X_1)/(G-1) \quad (1)$$

Where, d is the difference of the logarithms of the two adjacent concentration trials; X_K is the logarithm of the higher concentration; X_1 is the logarithm of the lower concentration; and G is the concentration trials.

$$\lg LC_{50}=X_K-d(\sum P-0.5) \quad (2)$$

Where X_K is the logarithm of the concentration with 100% mortality; d is the logarithmic interval; and $\sum P$ is the sum of the mortalities of all the trials (Xue *et al.*, 2005).

$$SC=(48 \text{ h } LC_{50} \times 0.3)/(24 \text{ h } LC_{50}/48 \text{ h } LC_{50})^2 \quad (\text{Zhang et al, 2011}) \quad (3)$$

According to the Environmental Safety Assessment of Agrochemical Test Guidelines provided by the Ministry of Environmental Protection of China (1989), the toxicities of agrochemicals to tadpoles were divided into low toxicity (LC₅₀>10.0 mg/L), moderate toxicity (LC₅₀=10.0–1.0 mg/L) and high toxicity (LC₅₀<1.0 mg/L) (Zhang *et al.*, 2010).

Joint toxicity evaluation

Marking's additive index of the coefficients for aquatic toxicology was used to evaluate joint toxicities (Xiu *et al.*, 1994).

$$S=(A_m/A_i)+(B_m/B_i) \quad (4)$$

Where, S is the joint toxicity of the paired agrochemicals; A and B are the experimental agrochemicals; A_i is the

LC₅₀ of agrochemical A when used alone; A_m is the LC₅₀ of agrochemical A when used jointly; B_i is the LC₅₀ of agrochemical B when used alone; and B_m is the LC₅₀ of agrochemical B when used jointly.

$$\text{When } S \leq 1, AI=1/S-1 \quad (5)$$

$$\text{When } S > 1, AI=-S+1 \quad (6)$$

When $AI < 0$, the coefficients were taken as antagonistic, when $AI > 0$, the coefficients were taken as synergistic, when $AI = 0$, the coefficients were taken as adding effects (Zhang *et al.*, 2011).

RESULTS

Acute toxicities of chlorantraniliprole, flubendiamide-abamectin and penoxsulam on tadpoles when used alone

When the agrochemicals were used individually, at the same exposure time point (24, 48 and 72 h), the mortalities of tadpoles increased with increasing concentrations and significant differences were found among the trials (one-way ANOVA, all $P < 0.001$, Table 1). The LC₅₀ of chlorantraniliprole, flubendiamide-abamectin and penoxsulam all decreased with increasing exposure time (24, 48 and 72 h), and their SCs were 1.23, 0.003, and 0.30 mg/L, respectively. Therefore, chlorantraniliprole showed moderate toxicity, and flubendiamide-abamectin and penoxsulam showed high toxicity (Table 2).

Joint toxicities of chlorantraniliprole, flubendiamide-abamectin and penoxsulam on tadpoles

When the agrochemicals were used in pairs, at the same exposure time point (24, 48 and 72 h), significant differences in the mortalities of tadpoles were also found among the trials (One-way ANOVA, all $P < 0.001$, Table 3). The LC₅₀ of the agrochemicals in each pair decreased with increasing exposure time. All the three joint pairs, chlorantraniliprole/penoxsulam, chlorantraniliprole/flubendiamide-abamectin and penoxsulam/flubendiamide-abamectin exhibited moderate toxicity and their LC₅₀ at 24, 48 and 72 h were 7.08, 6.61 and 6.03 mg/L; 2.455, 2.328 and 2.183 mg/L; and 1.132, 1.084 and 1.050 mg/L, respectively, whereas their corresponding SCs were 1.73, 0.63 and 0.30 mg/L, respectively (Table 2). Compared with the acute toxicity experiment, all paired LC₅₀ values decreased, except for that of penoxsulam in the chlorantraniliprole/penoxsulam pair. According to

Table 1 Acute toxicity for three agrochemicals on *H. chinensis* tadpoles ($n=10$)

Concentration (mg/L)	24 h mortality (%)	48 h mortality (%)	72 h mortality (%)
Chlorantraniliprole			
0.0	0.0±0.0 ^d	0.0±0.0 ^c	0.0±0.0 ^b
3.3	0.0±0.0 ^d	0.0±0.0 ^c	10.0±10.0 ^b
4.0	10.0±10.0 ^d	23.3±5.8 ^d	23.3±5.8 ^b
4.8	23.3±15.3 ^c	36.7±5.8 ^c	53.3±20.8 ^a
5.8	60.0±10.0 ^b	76.7±5.8 ^b	76.7±5.8 ^a
6.9	93.3±5.8 ^a	100.0±0.0 ^a	
8.3	100.0±0.0 ^a		
One-way ANOVA	$F_{6,14}=84.119^*$	$F_{5,12}=303.533^*$	$F_{4,10}=25.194^*$
Flubendiamide-abamectin			
0.000	0.0±0.0 ^d	0.0±0.0 ^d	0.0±0.0 ^b
0.013	0.0±0.0 ^d	0.0±0.0 ^d	6.7±5.8 ^b
0.019	23.3±5.8 ^c	36.7±5.8 ^c	60.0±20.0 ^a
0.029	36.7±5.8 ^c	53.3±5.8 ^b	70.0±17.3 ^a
0.044	56.7±15.3 ^b	90.0±10.0 ^a	95.0±7.1 ^a
0.066	83.3±5.8 ^a	100.0±0.0 ^a	
0.100	100.0±0.0 ^a		
One-way ANOVA	$F_{6,14}=96.467^*$	$F_{5,12}=199.200^*$	$F_{4,9}=26.657^*$
Penoxsulam			
0.0	0.0±0.0 ^c	0.0±0.0 ^d	0.0±0.0 ^d
0.8	0.0±0.0 ^c	0.0±0.0 ^d	0.0±0.0 ^d
1.0	13.3±5.8 ^{bc}	23.3±5.8 ^c	23.3±5.8 ^c
1.3	23.3±5.8 ^{bc}	46.7±15.3 ^b	60.0±0.0 ^b
1.6	30.0±0.0 ^b	50.0±0.0 ^{ab}	67.7±5.8 ^b
2.0	46.7±32.1 ^b	70.0±10.0 ^a	93.3±5.8 ^a
2.5	100.0±0.0 ^a		
One-way ANOVA	$F_{6,14}=23.222^*$	$F_{5,12}=40.309^*$	$F_{5,12}=241.333^*$

* $P<0.001$; Types with different superscripts differ significantly (Tukey's test, $\alpha=0.05$, $a>b>c>d>e$); NS: not significant.

Table 2 Half lethal concentration (LC₅₀) and safe concentration (SC) of the three agrochemicals administered individually and in conjunction

Pesticide	24 h	48 h	72 h	SC	Toxicity rank
Chlorantraniliprole	5.37	4.90	4.68	1.23	Moderately toxic
Flubendiamide-abamectin	0.035	0.025	0.021	0.003	Highly toxic
Penoxsulam	1.74	1.45	1.29	0.30	Moderately toxic
Chlorantraniliprole/Penoxsulam	7.08	6.61	6.03	1.73	Moderately toxic
Chlorantraniliprole/Flubendiamide-abamectin	2.455	2.328	2.183	0.63	Moderately toxic
Penoxsulam/Flubendiamide-abamectin	1.132	1.084	1.050	0.30	Moderately toxic

Marking's additive index, all agrochemical pairwise were antagonistic, except for that of chlorantraniliprole/flubendiamide-abamectin, which was synergistic at 24 h (Table 4).

Effects of agrochemical concentration, exposure time and their correlated effects on tadpole mortalities

According to the repeated measures ANOVA, both agrochemical concentration and exposure time significantly

Table 3 Toxicity of paired agrochemicals on *H. chinensis* tadpoles (n=10)

Concentration (mg/L)	24 h mortality (%)	48 h mortality (%)	72 h mortality (%)
Chlorantraniliprole/Penoxsulam			
0 (0/0)	0.0±0.0 ^d	0.0±0.0 ^d	0.0±0.0 ^d
5.62 (4.01/1.61)	0.0±0.0 ^d	0.0±0.0 ^d	23.3±5.8 ^c
6.17 (4.41/1.76)	13.3±5.8 ^c	30.0±0.0 ^c	63.3±5.8 ^b
6.76 (4.83/1.93)	20.0±0.0 ^c	46.7±5.8 ^b	86.7±5.8 ^a
7.41 (5.29/2.12)	83.3±5.8 ^b	90.0±10.0 ^a	100.0±0.0 ^a
8.13 (5.81/2.32)	93.3±5.8 ^{ab}	100.0±0.0 ^a	
8.91 (6.36/2.55)	100.0±0.0 ^a		
One-way ANOVA	$F_{6,14}=437.667^*$	$F_{5,11}=203.856^*$	$F_{4,9}=215.679^*$
Chlorantraniliprole/Flubendiamide-abamectin			
0 (0/0)	0.0±0.0 ^d	0.0±0.0 ^d	0.0±0.0 ^b
2.04 (2.027/0.013)	0.0±0.0 ^d	0.0±0.0 ^d	23.3±25.2 ^b
2.19 (2.176/0.014)	0.0±0.0 ^d	23.3±5.8 ^{cd}	60.0±0.0 ^a
2.34 (2.325/0.015)	36.7±30.6 ^{cd}	56.7±25.2 ^{bc}	80.0±10.0 ^a
2.51 (2.494/0.016)	60.0±17.3 ^{bc}	80.0±20.0 ^{ab}	90.0±0.0 ^a
2.69 (2.672/0.018)	86.7±5.8 ^{ab}	100.0±0.0 ^a	
2.88 (2.860/0.019)	100.0±0.0 ^a		
One-way ANOVA	$F_{6,14}=28.360^*$	$F_{5,12}=30.000^*$	$F_{4,9}=24.191^*$
Penoxsulam/Flubendiamide-abamectin			
0 (0/0)	0.0±0.0 ^c	0.0±0.0 ^d	0.0±0.0 ^c
0.95 (0.935/0.015)	0.0±0.0 ^c	0.0±0.0 ^d	13.3±5.8 ^{de}
1.02 (1.004/0.016)	10.0±10.0 ^{de}	26.7±25.2 ^{cd}	36.7±23.1 ^{cd}
1.09 (1.073/0.017)	36.7±15.3 ^{cd}	56.7±5.8 ^{bc}	63.3±5.8 ^{bc}
1.17 (1.151/0.019)	63.3±25.2 ^{bc}	83.3±5.8 ^{ab}	90.0±0.0 ^{ab}
1.25 (1.230/0.020)	83.3±5.8 ^{ab}	90.0±0.0 ^a	100.0±0.0 ^a
1.34 (1.319/0.021)	100.0±0.0 ^a		
One-way ANOVA	$F_{6,14}=35.378^*$	$F_{5,12}=41.076^*$	$F_{5,12}=49.789^*$

* $P<0.001$; Types with different superscripts differ significantly (Tukey's test, $\alpha=0.05$, a>b>c>d>e); NS: not significant.

Table 4 Evaluations of paired agrochemical toxicity

Pesticides	Exposure time (h)	LC ₅₀	S	AI	Toxicity evaluation
Chlorantraniliprole/Penoxsulam	24	7.08	2.105	-1.105	Antagonistic
	48	6.61	2.267	-1.267	Antagonistic
	72	6.03	2.261	-1.261	Antagonistic
Chlorantraniliprole/Flubendiamide-abamectin	24	2.455	0.942	0.062	Synergistic
	48	2.328	1.142	-0.142	Antagonistic
	72	2.183	1.282	-0.282	Antagonistic
Penoxsulam/ Flubendiamide-abamectin	24	1.132	1.174	-0.174	Antagonistic
	48	1.084	1.464	-0.464	Antagonistic
	72	1.050	1.644	-0.644	Antagonistic

affected the mortalities of tadpoles. All trials showed significantly correlated effects on the mortalities of tadpoles, except for the flubendiamide-abamectin/penoxsulam pair (Table 5).

DISCUSSION

This study evaluated the acute and joint toxicities of chlorantraniliprole, flubendiamide-abamectin and penoxsulam to tiger frog tadpoles. Chlorantraniliprole has been

Table 5 Effects of agrochemical concentration, exposure time and their interactions on mortality of *H. chinensis* tadpoles

Pesticide	Concentration	Exposure time	Concentration×Exposure time
Chlorantraniliprole	$F_{5,12}=103.159^{**}$ I ^e , II ^{de} , III ^c , IV ^c , V ^b , VI ^a	$F_{2,24}=31.462^{**}$ 24h ^c , 48h ^b , 72h ^a	$F_{10,24}=4.323^*$
Flubendiamide-abamectin	$F_{5,12}=133.302^{**}$ I ^d , II ^c , III ^c , IV ^b , V ^{ab} , VI ^a	$F_{2,24}=38.603^{**}$ 24h ^c , 48h ^b , 72h ^a	$F_{10,24}=4.508^*$
Penoxsulam	$F_{5,12}=76.592^{**}$ I ^d , II ^d , III ^c , IV ^c , V ^b , VI ^a	$F_{2,24}=40.831^{**}$ 24h ^c , 48h ^b , 72h ^a	$F_{10,24}=4.720^*$
Chlorantraniliprole/Penoxsulam	$F_{5,12}=645.760^{**}$ I ^e , II ^d , III ^c , IV ^b , V ^{ab} , VI ^a	$F_{2,24}=195.470^{**}$ 24h ^c , 48h ^b , 72h ^a	$F_{10,24}=30.510^{**}$
Chlorantraniliprole/Flubendiamide-abamectin	$F_{5,12}=43.905^{**}$ I ^d , II ^{cd} , III ^b , IV ^{ab} , V ^a , VI ^a	$F_{2,24}=28.015^{**}$ 24h ^c , 48h ^b , 72h ^a	$F_{10,24}=2.686^*$
Penoxsulam/Flubendiamide-abamectin	$F_{5,12}=53.116^{**}$ I ^c , II ^c , III ^b , IV ^a , V ^a , VI ^a	$F_{2,24}=22.548^{**}$ 24h ^c , 48h ^b , 72h ^a	$F_{10,24}=1.671^{NS}$

*: $P<0.05$; **: $P<0.001$; I, II, III, IV, V and VI: six different concentrations of each agrochemical (see details in materials and methods); Types with different superscripts differ significantly (Tukey's test, $\alpha=0.05$, a>b>c>d>e); NS: not significant.

used for rice pest control in China since 2008. The LC_{50} of chlorantraniliprole in our study differed from previous findings. Huang et al (2011) found that chlorantraniliprole exhibited low toxicity in controlling *Chilo suppressalis* at different developmental stages and its LC_{50} was between 2.00 and 18.70 ng. Barbee et al (2010) also found low toxicity ($LC_{50}=951 \mu\text{g/L}$ at 96 h) in an acute toxicity experiment on *Procambarus clarkii*, and no effects from chlorantraniliprole on either the mortalities or behaviors of *P. clarkii* were found. In our study, the LC_{50} of chlorantraniliprole on tiger frog tadpoles at 24, 48, and 72 h were 7.08, 6.61, and 6.03 mg/L, respectively, which were much higher than those on *C. suppressalis* and *P. clarkii*. According to the Environmental Safety Assessment of Agrochemical Test Guidelines, chlorantraniliprole showed moderate toxicity to tiger frog tadpoles.

Flubendiamide-abamectin has been available since 2010 for *C. suppressalis* control. Lin (2012) reported it as highly toxic, with 99.64% mortality on *C. suppressalis* (10%, 30 mL/667 m²). Our study also showed that it was highly toxic, with LC_{50} values at 24, 48 and 72 h of 0.035, 0.025 and 0.021 mg/L, respectively.

Penoxsulam is a broad-spectrum weedicide, used to control species such as *Myriophyllum heterophyllum* (Glomski & Netherland, 2008), but its toxicity on animals is little understood. Our study found that penoxsulam exhibited moderate toxicity, with LC_{50} values at 24, 48 and 72 h of 1.74, 1.45 and 1.29 mg/L, respectively.

Our results showed that the toxicities of the three

agrochemicals, from high to low, were flubendiamide-abamectin, penoxsulam and chlorantraniliprole, with SCs of 0.003, 0.30, and 1.23 mg/L, respectively (Table 2). Concentration, exposure time, and their interaction effects had significant influences on the mortalities of tiger frog tadpoles, except for the flubendiamide-abamectin/penoxsulam pair (Table 5).

Previous studies demonstrated that the joint toxicities of different agrochemicals and heavy metals on amphibians, such as *Bufo gargarizans* (Zhang et al, 2010) and *Rana limnocharis* (Guo et al, 2005; Nian et al, 2009), are usually synergistic. In our joint toxicity experiment, at same exposure time, all concentration trials of each pair had significant effects on the mortalities of tadpoles (Table 3). The LC_{50} of the chlorantraniliprole/penoxsulam pair after 24, 48, and 72 h were 7.08, 6.61, and 6.03 mg/L, respectively, whereas, their individual LC_{50} when used jointly were 5.01/2.04, 4.68/1.91, and 4.27/1.74 mg/L, respectively. The LC_{50} of chlorantraniliprole in this pair was lower than that in the acute experiment; however, the situation was opposite for penoxsulam (Table 2, 4). This pair was antagonistic in toxicity evaluation (Table 4).

The LC_{50} of the chlorantraniliprole/flubendiamide-abamectin pair after 24, 48 and 72 h were 2.455, 2.328 and 2.183 mg/L, respectively, whereas, their individual LC_{50} when used jointly were 2.45/0.017, 2.34/0.0166, and 2.32/0.0165 mg/L, respectively. The LC_{50} of both agrochemicals were lower than those in the acute experiment (Table 2, 4). This pair was synergistic at 24 h, but antagonistic at 48 and 72 h in toxicity evaluation (Table 4).

The LC₅₀ of the penoxsulam/flubendiamide-abamectin pair after 24, 48 and 72 h were 1.132, 1.084 and 1.050 mg/L, respectively, whereas, their individual LC₅₀ when used jointly were 1.114/0.0187, 1.067/0.0182, and 1.033/0.0177 mg/L, respectively. The LC₅₀ of both agrochemicals were lower than those in the acute experiment (Table 2, 4). This pair was antagonistic in toxicity evaluation (Table 4). As a result, the response of tiger frog tadpoles to these agrochemicals (individually or jointly) is complicated as these antagonistic phenomena are correlated with animal developmental stage, as well as exposure time and concentration of chemicals (Berrill *et al.*, 1993).

Amphibians play important roles in pest control and in the aquatic and terrestrial food webs. However, due to environmental and habitat degeneration, amphibian

resources have declined dramatically in recent years. Agrochemicals can change both the quantity and quality of amphibian habitats and food resources (Berrill *et al.*, 1993), and induce mutations during development, such as retardation and deformation, as well as abnormal behaviors (Pawar *et al.*, 1983; Sayim, 2008). Lethal and or sub-lethal toxicities of agrochemicals might affect the natural adjustment procedures of amphibian species (especially newborn or juvenile groups), and may help explain population decline. The rational application of agrochemicals is, therefore, critical for environmental and amphibian protection.

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