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Acute Toxicity of the Pesticide Dichlorvos and the Herbicide Butachlor to Tadpoles of Four Anuran Species

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Amphibian population declines exceeding normal population fluctuations have been reported globally in recent years and extinction has occurred in some populations (Carey and Bryant 1995; Daszak et al. 1999; Stuart et al. 2004). Although no single cause has been identified, current studies raise concern regarding the potential role that pesticides may play in these declines (Sparling et al. 2001; Xu et al. 2003). Chemical contamination as a consequence of pesticide application continues to be postulated as a contributing factor in the global decline of amphibian populations (Ankley et al. 1998; Mann and Bidwell 2001). Indeed, amphibians may be at greater risk from the toxic effects of pesticides than other aquatic vertebrates because their preferred breeding habitats are often shallow, lentic or ephemeral water bodies (Tyler 1994) where contaminants may accumulate without substantial dilution.

Dichlorvos and butachlor are the most commonly used pesticide and herbicide, respectively in China, and are applied to field crops, fruits and vegetables, so their potential toxicity is of particular interest. More than 10,000 tons (10^7 kg) of each are produced annually in China and increased production is expected in the future (Hu 1998; Yao et al. 1999).

Dichlorvos is an organophosphate pesticide. The active compound is 2, 2 – Dichlorvinyl dimethylphosphate (Chemical Formula: $C_4H_7Cl_2O_4P$, m.w. 220.98) (WHO 1989). It has been used as pesticide since 1955 and is now widely used to kill insect pests in farming, forestry, and the general environment. Dichlorvos can kill insects when ingested, absorbed through the integument or spiracles (Worthing 1991). It is effective against a wide range of pests such as mushroom flies, aphids, spider mites, caterpillars, thrips, whiteflies, gypsy moths, spruce budworms, forest tent caterpillars, fruit flies, codling moths, corn borers and boll weevils (Meister 1998). The hydrolization rate is 3% per day (Institute of Plant Protection, Chinese Academy of Agricultural Sciences 1988). Butachlor is a widely used herbicide in Asia and South America to control a wide range of

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annual grasses and some broadleaf weeds, and is applied either pre-emergence or early post-emergence. 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. The active compound is N - Butoxymethyl - a - chloro - 2 - 6 diethylacetanilide (Chemical Formula: $C_{17}H_{26}ClNO_2$, m.w. 311.86). The half-life is 1.65-2.48 days in field water and 2.67-5.33 days in the soil (Yu et al. 1993).

Because of their frequency of application and broad array of uses, the potential negative impacts of these two agents need to be carefully considered. Accurate diagnosis of the cause of amphibian decline is essential before steps can be taken to slow or halt the process (Christy and Dickman 2002; Caughley 1994). The toxicity of dichlorvos and butachlor to tadpoles of four species of frog was tested in this study, to discern whether or not these agents might be contributing to amphibian declines.

MATERIALS AND METHODS

Dichlorvos (80%, Emulsion) was provided by the Dachen Pesticides Factory, Shandong Province. Butachlor (50%, Emulsion) was provided by Qingfeng Pesticides and Chemical Product Corporation, Hangzhou, Zejian Province. Commercial preparations of the agents were tested because it is these forms to which organisms are exposed in their natural environment. Prior to testing, a primary stock was prepared of each test substance at a nominal concentration of 1 ml/L. The diluent used for the stock solutions was water with a hardness of 200 mg/L $CaCO_3$ and a conductivity of approximately 98 μs/cm. Test concentrations were made up using this water immediately prior to the beginning of the tests.

Four species of anuran (*Bufo melanostictus, Fejervarya multistriata, Polypedates megacephalus* and *Microhyla ornata*) were used in this study. The four species are widely distributed in south China and serve as examples of four of the major phylogenetic groups of anuran in China (Bufonidae, Ranidae, Rhacophoridae, Microhylidae); they are also typical representatives of medium to small-sized anurans. *B. melanostictus* is a medium sized (76-106 mm snout-vent length [s-v]) toad. *F. multistriata* is a small (40–46mm s-v) frog. *P. megacephalus* is a medium-sized (53-76mm s-v) treefrog. *M. ornata* is a small (22-23mm s-v) narrow-mouthed frog. All four species principally inhabit agricultural and urban landscapes, and their tadpoles inhabit water bodies. Their reproductive periods are relatively long (March to August for *B. melanostictus* and *M. ornata*; March to September for *F. multistriata*; April to August for *P. megacephalus*) (Cai 1979), so the chances of their tadpoles coming into contact with dichlorvos and butachlor are very high.

Adults of each species were collected from an agricultural field in a suburb of Fuzhou city, Fujian Province, China. These adults were placed in an artificial pond at our College that is drained and re-filled with clean water annually prior to the beginning of spring. The fertilized egg masses were collected and reared to tadpole stage (Gosner-stage) 25-26 in the laboratory (Gosner 1960). Tadpoles were used in the study because they are sensitive to pollution at an early stage in their development.

All tadpoles were held in glass tanks for 48 h in water and fed with eel fodder. After 5 ~ 7 days of acclimation, healthy tadpoles at the same stage for each species were selected for the toxicity tests. Total body lengths (including the tail) were monitored prior to testing (Table 1).

Static-renewal acute toxicity tests were conducted at room temperature (water temperature 21-27℃), similar to that to which the organisms are exposed in their natural environment. Four to eight equal logarithm interval concentrations (0.0625 for *B. melanostictus and P. megacephalus*; 0.125 for *F. multistriata* and *M. ornata*) and a control were used for these tests. 2000 ml basins with 1000 ml of solution were used for all the tests. For all four species, ten tadpoles were randomly allocated to each test concentration and a control group. Animals were not fed for the 96-h duration of the tests. Test solutions were renewed after 24 h. Animal condition was assessed and dead animals were removed and the number dead recorded at 24-h intervals. The method of probabilitistic-logarithmic transformation was used to determine the LC50 at 24-h, 48-h, 72-h and 96-h, respectively. Data are from duplicate basins. All data processing was carried out using the statistical software package SPSS 11.0.

RESULTS AND DISCUSSION

The LC50 values at 24, 48, 72 and 96-h are presented in Table 1. The acute toxicity to frogs of these classes of chemicals appears to be substantially related to their ability to induce death in aquatic organisms. Dichlorvos LC50 values for the four species were between 0.78 mg/L (96-h) for *M. ornata* and 78.93 mg/L (48-h) for *B. melanostictus*. Dichlorvos produced similar LC50s in two species, *F. multistriata* and *P. megacephalus*. For tadpoles exposed to butachlor, all LC50 values for the four species ranged between 0.53 mg/L (96-h) for *M. ornata* to 2.67 mg/L (24-h) for *P. megacephalus*.

Differences in the sensitivities of these frog species to these chemicals were evident. The reasons for these differences remain unknown. However, we suspect

Table 1. 24, 48, 72 and 96-h LC50 values with 95% confidence intervals (CI) for acute toxicity tests with Gosner-stage 25-26 tadpoles of four species of frogs exposed to dichlorvos and butachlor in static-renewal tests at water temperature 21-27℃

Species	Body length (mean ±SD; mm)	Exposed time (h)	LC50 (95%CI; mg/L)	
			Dichlorvos	Butachlor
B. melanostictus	12.93±0.78	24		1.70 (1.50~1.93)
		48	78.93 (73.02~85.31)	1.18 (1.06~1.31)
		72	54.91 (50.83~59.32)	
		96	51.64 (47.94~55.63)	
F. multistriata	14.79±1.11	24	57.38 (47.18~69.80)	
		48	21.27 (17.84~25.36)	1.46 (1.25~1.70)
		72	12.83 (10.16~16.20)	1.36 (1.10~1.67)
		96	10.53 (8.80~12.59)	1.30 (1.16~1.45)
P. megacephalus	16.46±1.31	24	44.78 (33.06~60.67)	2.67 (2.36~3.02)
		48	26.41 (23.59~29.57)	2.62 (2.29~3.01)
		72	17.45 (15.24~19.97)	1.70 (1.34~2.15)
		96	12.94 (10.10~16.56)	1.52 (1.23~1.87)
M. ornata	6.18±0.41	24	3.13 (2.12~4.61)	1.69 (1.46~1.95)
		48	1.58 (1.14~2.18)	0.85 (0.71~1.01)
		72		0.61 (0.52~0.71)
		96	0.78 (0.41~1.47)	0.53 (0.45~0.61)

that the different sensitivities are related to tadpole size, differences of life history, and the type of water normally inhabited by the tadpoles.

First, the sensitivity of the frogs to toxicity is related to the size of the animal. The larger the body size, the stronger the resistance to the effects of the pesticide and/or the herbicide. *M. ornata* was the most sensitive species to dichlorvos, while *B. melanostictus* was the least sensitive. *M. ornata* was the most sensitive species to butachlor, while *P. megacephalus* was the least sensitive.

Second, the sensitivities to toxicity are related to life history differences among species. The longer the tadpole stage and the time in water bodies, the greater the toxic impact. The embryonic and tadpole stages of *M. ornata* are relatively short (Fei and Ye 1983; Geng et al. 1996). Although it is the most sensitive species to the pesticide and the herbicide, its populations in agricultural fields are still enormous (Wang and Xie 2004). Their tadpoles can metamorphose and move away from the water bodies before the water becomes too polluted.

Third, the sensitivity to toxicity is related to the type of water normally inhabited by the tadpoles. The dirtier the water inhabited by the tadpoles, the stronger the resistance of the tadpoles to the effects of the chemical agents. *B. melanostictus* tadpoles displayed lower sensitivity to dichlorvos than the other three species. This stronger resistance is probably in part related to the dirtier habitats that are used. *B. melanostictus* belongs to Bufonidae, the species of which often inhabit dirty, shallow, or lentic water bodies and can be found in and around areas of garbage and refuse (Cai 1979), where contaminants can accumulate without substantial dilution. However, the habitats of *F. multistriata, P. megacephalus* and *M. ornata* are comparatively clean, and so these species have relatively lower resistance to pollution.

Pesticides and herbicides have previously been implicated in the decline and extinction of some amphibian populations (Sparling et al. 2001). Tighter controls on the release and application of pesticides and herbicides can probably make a significant contribution to the protection of amphibian populations.

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